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JC923 U.S. PTO  
09/678023  
10/04/00**UTILITY  
PATENT APPLICATION  
TRANSMITTAL**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.

5.1158 Div. I

First Named Inventor or Application Identifier

Hideki Kawasaki

Express Mail Label No.

**APPLICATION ELEMENTS**

See MPEP chapter 600 concerning utility patent application contents.

**ADDRESS TO:**Commissioner for Patents  
Box Patent Application  
Washington, DC 20231

1. ☐ Fee Transmittal Form  
(Submit an original, and a duplicate for fee processing)
2. ☐ Applicant claims small entity status.  
See 37 CFR 1.27.
3. ☒ Specification *Total Pages* **46**
4. ☒ Drawing(s) (35 USC 113) *Total Sheets* **2**
5. ☒ Oath or Declaration *Total Pages* **2**
- a. ☐ Newly executed (original or copy)
- b. ☒ Copy from a prior application (37 CFR 1.63(d))  
(for continuation/divisional with Box 17 completed)  
[Note Box 6 below]
- i. ☐ **DELETION OF INVENTOR(S)**  
Signed Statement attached deleting  
inventor(s) named in the prior application, see  
37 CFR 1.63(d)(2) and 1.33(b).
6. ☒ Application Data Sheet. See 37 CFR 1.76

7. ☐ CD-ROM or CD-R in duplicate, large table or Computer  
Program (Appendix)
8. ☐ Nucleotide and/or Amino Acid Sequence Submission  
(if applicable, all necessary)
- a. ☐ Computer Readable Form (CRF)
- b. Specification Sequence Listing on:
- i. ☐ CD-ROM or CD-R (2 copies); or
- ii. ☐ paper
- c. ☐ Statements verifying identity of above copies

**ACCOMPANYING APPLICATION PARTS**

9. ☐ Assignment Papers (cover sheet & document(s))
10. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attorney  
(when there is an assignee)
11. ☐ English Translation Document (if applicable)
12. ☒ Information Disclosure ☐ Copies of IDS  
Statement (IDS)/PTO-1449 Citations
13. ☒ Preliminary Amendment
14. ☒ Return Receipt Postcard (MPEP 503)  
(Should be specifically itemized)
15. ☐ Certified Copy of Priority Document(s)  
(if foreign priority is claimed)
16. ☐ Other: \_\_\_\_\_

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:



Continuation



Divisional



Continuation-in-part (CIP)

of prior application No. 08 / 894,344

Prior application information:

Examiner Peter Tung, Ph.D.Group/Art Unit: 1652

For CONTINUATION OR DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 5b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

**18. CORRESPONDENCE ADDRESS**

Customer Number or Bar Code Label

**05514**

(Insert Customer No. or Attach bar code label here)



Correspondence address below

NAME

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| CLAIMS | (1) FOR  | (2) NUMBER FILED | (3) NUMBER EXTRA              | (4) RATE                      | (5) CALCULATIONS |
|--------|--|------------------|-------------------------------|-------------------------------|------------------|
|        | TOTAL CLAIMS<br>(37 CFR 1.16(c))   | 23 -20 =         | 3                             | X \$ 18.00 =                  | \$54.00          |
|        | INDEPENDENT CLAIMS (37 CFR 1.16(b))  | 3 -3 =           | 0                             | X \$80.00 =                   | \$0.00           |
|        | MULTIPLE DEPENDENT CLAIMS (if applicable) (37 CFR 1.16(d))                 |                  |                               | \$270.00 =                    | \$270.00         |
|        |  |                  |                               | BASIC FEE<br>(37 CFR 1.16(a)) | \$710.00         |
|        |  |                  | Total of above Calculations = |                               | \$1034.00        |
|        | Reduction by 50% for filing by small entity (Note 37 CFR 1.9, 1.27, 1.28). |                  |                               |                               |                  |
|        | TOTAL =  |                  |                               |                               | \$1034.00        |

19. Small entity status

- a. ☐ A small entity statement is enclosed
- b. ☐ A small entity statement was filed in the prior nonprovisional application and such status is still proper and desired.
- c. ☐ Is no longer claimed.

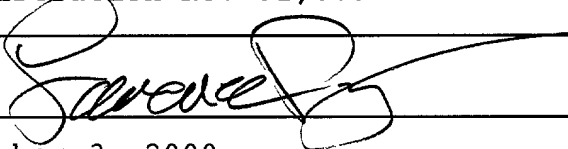
20. ☒ A check in the amount of \$ 1034.00 to cover the filing fee is enclosed.

21. ☐ A check in the amount of \$ \_\_\_\_\_ to cover the recordal fee is enclosed.

22. The Commissioner is hereby authorized to credit overpayments or charge the following fees to Deposit Account No. 06-1205:

- a. ☒ Fees required under 37 CFR 1.16.
- b. ☒ Fees required under 37 CFR 1.17.
- c. ☐ Fees required under 37 CFR 1.18.

**SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED**

|           |  |
|-----------|--|
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| SIGNATURE |  |
| DATE      | October 3, 2000  |

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#### CORRESPONDENCE INFORMATION

Correspondence Customer Number:: 05514  
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#### APPLICATION INFORMATION

Title Line One:: NOVEL YEAST GENE

Total Drawing Sheets:: 2  
Formal Drawings?: Yes  
Application Type:: Utility  
Docket Number:: 5.1158 DIV I  
Secrecy Order in Parent Appl.?: No

00400T" E2082960

REPRESENTATIVE INFORMATION

Representative Customer Number:: 5514

PRIOR FOREIGN APPLICATIONS

Foreign Application One:: 343700/95

Filing Date:: December 12, 1995

Country:: Japan

Priority Claimed:: Yes

NY\_MAIN 116169 v 1

004007" E003/950

5.1158 DIV. I

PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: )  
HIDEKI KAWASAKI, ET AL. )  
Application No.: (Divisional )  
of Serial No. 08/894,344 )  
filed August 15, 1997) )  
Filed: Currently herewith )  
For: NOVEL YEAST GENE )

Examiner: Peter Tung, Ph.D.  
Group Art Unit: 1652  
October 3, 2000

Assistant Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to action on the merits, please amend the  
above-identified application as follows:

IN THE TITLE:

Please amend the Title to read: --PROTEIN  
COMPLEMENTING YEAST LOW TEMPERATURE-SENSITIVITY  
FERMENTABILITY--.

IN THE SPECIFICATION:

Page 1, line 3, add --This application is a  
division of application No. 08/894,344 filed  
August 15, 1997--.

Page 10, line 14, change "lowered" to --improved--.

IN THE CLAIMS:

Please cancel Claim 1 and 2.

Please amend claims 6 and 8-10 as follows:

Claim 6, line 1, delete "or 5".

Claim 8, lines 1-2, change "any one of Claims 4-7"  
to --Claim 4--.

Claim 9, line 2, change "any one of claims 4-7" to  
--Claim 4--.

Claim 10, line 2, change "any one of claims 4-7" to  
--Claim 4--.

004007 "E2082950

Please add the following new Claims 11-14:

--11. The yeast according to Claim 5, wherein the sequence at positions 4388 through 7885 in the nucleotide sequence represented by SEQ ID NO: 1 is disrupted.

12. Dough containing the yeast according to Claim 11.

13. A process for making bread which comprises adding the yeast according to Claim 11 to dough.

14. A process for producing ethanol which comprises culturing the yeast according to Claim 11 in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.--

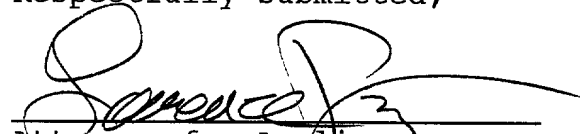
#### REMARKS

The specification has been amended to correct an inadvertent clerical error. The claims have been amended and new Claims 11-14 added to maintain their dependency in conformity with accepted U.S. practice. No new matter has been added.

Entry hereof is earnestly solicited.

Applicants undersigned attorney may be reached in our New York office by telephone at (212) 218-2100. All correspondence should continue to be directed to our below listed address.

Respectfully submitted,



Attorney for Applicant  
Lawrence S. Perry  
Registration No. 31,865

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SPECIFICATION  
NOVEL YEAST GENE

Technical Field

5           The present invention relates to a process for making bread with refrigerated dough and a process for producing ethanol.

Background Art

10           Recently, in the bread manufacturing industry, a method for making bread with refrigerated dough has been widely used with the purpose of saving labor in the bread making process and meeting diverse needs of consumers. In this method, partially fermented dough is stored at a low  
15           temperature in a refrigerator and then is subjected to fermentation, proofing and baking to make bread. Such a method is usually carried out by the use of refrigeration-resistant yeast, that is, yeast which is capable of controlling fermentation during the storage of dough at a  
20           low temperature and allowing normal fermentation at temperatures for fermentation and proofing to raise the dough.

As for the breeding of refrigeration-resistant yeast, there are known methods in which yeast strains of wild type  
25           are conferred with the mutation exhibiting low-temperature-sensitive fermentability by artificial mutagenesis [e.g., Japanese Published Examined Patent Application No. 71474/95, Japanese Published Unexamined Patent Application No. 213277/95, Japanese Published Unexamined Patent  
30           Application No. 79767/95, and Appl. Environ. Microbiol., 61, 639-642 (1995)]. The yeast strains conferred with the mutation exhibiting low-temperature-sensitive fermentability are used as refrigeration-resistant yeast or as parent strains for breeding refrigeration-resistant  
35           yeast.

However, such mutagenesis induces mutation at random

and thus may possibly confer the yeast with mutation relating to the basic properties of fermentation such as dough raising, in addition to the low-temperature-sensitivity mutation.

5 It is also known to confer baker's yeast or brewer's yeast with favorable properties such as flocculation [The 23rd European Brewery Conv. Proc., 297-304 (1991)] and flavor [Curr. Genet., 20, 453-456 (1991)] by using gene manipulation techniques.

10 However, a gene relating to the low-temperature-sensitivity of fermentability or a method for breeding refrigeration-resistant yeast by gene manipulation is not known.

Ethanol is produced by fermentation of sugar materials  
 15 (e.g. molasses) or starch materials (e.g. corn and potato) as carbon sources. Fermentation can be generally carried out at a temperature of 30 to 43°C. Usually, the fermentation temperature is adjusted to 30 to 35°C by cooling in order to avoid the death, insufficient growth,  
 20 or decrease in fermentability of yeast caused by the rise of temperature. However, in the summer months, cooling is often insufficient, thereby causing the rise of culturing temperature to 35 to 38°C in the course of alcohol fermentation. Thus, alcohol fermentation is usually  
 25 carried out with further cooling to prevent the rise of temperature due to fermentation heat. A need exists for temperature-resistant yeast which is useful for saving cost for cooling in such process.

As for the breeding of thermotolerant yeast, there  
 30 have been reports on a method in which mitochondria relating to thermotolerance is introduced [Juan Jimenez, et al.: Curr. Genet., 13, 461-469 (1988)] and a method in which heat shock protein HSP104 is expressed at a high level [Susan Lindquist, et al.: Proc. Natl. Acad. Sci. USA,  
 35 93, 5301-5306 (1996)]. However, application of these methods to alcohol fermentation has not been studied. Further, it is known that the heat-resistance of yeast is

improved by heat treatment at temperatures which are not fatal to the yeast [B.G. Hall: J. Bacteriol., 156, 1363 (1983)], but this effect is not lasting, and it is difficult to apply this method to alcohol fermentation.

5

#### Disclosure of the Invention

The present invention relates to a protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence represented by SEQ ID NO: 1; a gene which encodes said protein; and a gene which comprises DNA having the nucleotide sequence represented by SEQ ID NO: 1, or comprises DNA being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having a nucleotide sequence wherein one or more DNAs are added, deleted or substituted in the nucleotide sequence represented by SEQ ID NO: 1. The present invention also relates to yeast belonging to the genus Saccharomyces and having low-temperature-sensitive fermentability which is characterized in that the above-mentioned gene on the chromosome is inactivated; dough containing said yeast; a process for making bread which comprises adding said yeast to dough; and a process for producing ethanol which comprises culturing said yeast in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.

The expression "having low-temperature-sensitive fermentability" as used herein means the property of having substantially no fermentability at temperatures for low temperature storage and having normal fermentability at temperatures for fermentation and proofing after the low temperature storage. For instance, in the case of baker's yeast, it means the property of having substantially no dough-raising ability at 5°C and having normal dough-

raising ability at 20 to 40°C after the storage under refrigeration at 5°C for 1 to 7 days, and in the case of brewer's yeast, it means the property of having substantially no alcohol fermentability at 5°C and having normal alcohol fermentability at 20 to 40°C after the storage under refrigeration at 5°C for 1 to 7 days.

Isolation of a gene which complements the mutation exhibiting low-temperature-sensitive fermentability, determination of the DNA sequence of said gene, and inactivation of said gene can be carried out by using basic techniques for genetic engineering and biological engineering according to the descriptions in commercially available experiment manuals, e.g. Gene Manual, Kodansha Co., Ltd.; Methods for Experiments in Gene Manipulation, edited by Yasutaka Takagi, Kodansha Co., Ltd.; Molecular Cloning, Cold Spring Harbor Laboratory (1982); Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory (1989); Methods in Enzymology, 194 (1991); and Gene Experiments Using Yeasts (an extra number of Experimental Medicine), Yodosha Co., Ltd. (1994).

The gene which complements the mutation exhibiting low-temperature-sensitive fermentability according to the present invention (hereinafter referred to as the gene complementing low-temperature-sensitivity) can be isolated, for example, as the gene complementing the low-temperature-sensitivity of fermentability of Saccharomyces cerevisiae RZT-3 (FERM BP-3871) (hereinafter referred to as RZT-3 strain) described in Japanese Published Unexamined Patent Application No. 336872/93. That is, the gene complementing low-temperature-sensitivity can be isolated by transforming RZT-3 strain with the DNA library of the yeast carrying the gene complementing low-temperature-sensitivity, and obtaining DNA from the strain of which the mutation exhibiting low-temperature-sensitive fermentability is complemented.

The DNA library of the yeast carrying the gene complementing low-temperature-sensitivity can be prepared

by cleaving the chromosomal DNA of yeast carrying a gene of wild type, e.g. Saccharomyces cerevisiae X2180-1B

(hereinafter referred to as X2180-1B strain) with a restriction enzyme, and ligating each of the obtained DNA fragments with a vector capable of being maintained in yeast.

Any restriction enzymes which can cleave the chromosomal DNA can be used in the above process. Preferably, those which give DNA fragments of 20 Kbp or less are used. The chromosomal DNA may be completely digested or partially digested with the restriction enzyme.

Examples of the vectors capable of being maintained in yeast are YCp vectors, YEp vectors, YRp vectors, YIp vectors, and YAC (yeast artificial chromosome) vectors.

The transformation of RZT-3 strain with the DNA library can be carried out according to the methods generally used in genetic engineering and biological engineering such as the spheroplast method [e.g. Proc. Natl. Acad. Sci. USA, 75, 1929-1933 (1978)], the lithium acetate method [e.g. J. Bacteriol, 153, 163-168 (1983)], and the electroporation method [e.g. Methods in Enzymology, 194, 182-187 (1991)].

The complementation of the mutation exhibiting low-temperature-sensitive fermentability can be confirmed by examining the transformed yeast for the growth at a low temperature or the fermentability at a low temperature [Appl. Environ. Microbiol., 61, 639-642 (1995)]. The examination on fermentability at a low temperature can be carried out, for example, by the pigment agar layer method described below. In this method, the test strain is cultured at 30°C on YPG agar medium (1% yeast extract, 2% peptone, 3% glycerol, and 2% agar) to form colonies. Then, a pigment agar (0.5% yeast extract, 1% peptone, 10% sucrose, 0.02% Bromocresol Purple, and 1% agar, pH 7.5) is layered over the medium, and the plate is kept at a low temperature (e.g. 5°C). Bromocresol Purple is a pH

indicator, and the pigment agar assumes a purple color when being layered. Fermentation of the yeast lowers the pH of the medium around the colony, thereby causing the change of the color of that area from purple to yellow. Accordingly, a strain showing the color change to yellow around the colony while the layered plate is kept at a low temperature can be selected as a strain having fermentability at a low temperature.

Recovery of a plasmid from the yeast and transformation of Escherichia coli using the plasmid can be carried out according to the methods generally used in genetic engineering. For example, the plasmid can be recovered by the method described in Gene Experiments Using Yeasts (an extra number of Experimental Medicine), Yodosha Co., Ltd. (1994), and the transformation can be carried out by the method described in Molecular Cloning, 2nd ed., Cold Spring Harbor Laboratory (1989).

The nucleotide sequence of the gene complementing low-temperature-sensitivity can be determined by the methods generally used in genetic engineering such as the Maxam-Gilbert method and the dideoxy method.

The polypeptide encoded by the gene complementing low-temperature-sensitivity can be readily obtained by using current knowledge of molecular genetics. If necessary, analysis using computers can be made [e.g. Cell Technology, 14, 577-588 (1995)]. It is possible to use the polypeptide encoded by the gene complementing low-temperature-sensitivity as an inhibitor to the low-temperature-sensitivity of fermentability in the yeast having low-temperature-sensitive fermentability.

The present invention has clarified the nucleotide sequence of the gene complementing low-temperature-sensitivity and the amino acid sequence of the polypeptide encoded by the gene, and thereby has enabled disruption of the gene complementing low-temperature-sensitivity, regulation of expression or alteration of expression level of the gene complementing low-temperature-sensitivity by

modification of the promoter, expression of various genes by the use of the promoter of the gene complementing low-temperature-sensitivity, preparation of a fused gene in which the gene complementing low-temperature-sensitivity is fused with another gene as well as a fused polypeptide, and the like. These manipulations can be carried out by using, for example, the methods described in Methods in Enzymology, 194, 594-597 (1991).

The methods for inactivating the gene complementing low-temperature-sensitivity in yeast are described below.

The term inactivation of the gene as used herein refers to the lowering or loss of functions inherent in the gene or the polypeptide encoded by the gene induced by various techniques for genetic engineering or biological engineering; for example, gene disruption [e.g. Methods in Enzymology, 194, 281-301 (1991)], introduction of a movable genetic element into the gene [e.g. Methods in Enzymology, 194, 342-361 (1991)], introduction and expression of the antisense gene [e.g. Japanese Published Examined Patent Application No. 40943/95, and The 23rd European Brewery Conv. Proc., 297-304 (1991)], introduction of DNA relating to silencing to the vicinity of the gene [e.g. Cell, 75, 531-541 (1993)], and treatment of the polypeptide encoded by the gene with an antibody [e.g. European J. Biochem., 231, 329-336 (1995)].

For the inactivation of the gene complementing low-temperature-sensitivity, any yeast which belongs to the genus Saccharomyces, preferably Saccharomyces cerevisiae, can be used. That is, various kinds of yeasts such as baker's yeast, sake yeast, wine yeast, beer yeast, miso and soy sauce yeast, and ethanol-producing yeast belonging to the genus Saccharomyces can be used.

The disruption of the gene complementing low-temperature-sensitivity means a process which comprises introducing into yeast cells DNA which has a nucleotide sequence homologous to that of the gene complementing low-

temperature-sensitivity but is incapable of acting as the gene complementing low-temperature-sensitivity due to a mutation such as addition, deletion or substitution, to induce homologous recombination, and thereby incorporating this mutation into the gene on the genome.

The DNA used for the gene disruption can be prepared, for example, by a method which comprises cleavage of the gene complementing low-temperature-sensitivity with restriction enzymes to add, delete or substitute DNAs, and a method which comprises extracellular mutation (in vitro mutagenesis) of the gene complementing low-temperature-sensitivity. For the addition and substitution of DNAs, a method can be used in which the marker gene is inserted.

The disruption of the gene complementing low-temperature-sensitivity can be effected by disruption of any of the promoter region, open reading frame region, and terminator region of the gene, or combinations of such regions. The gene complementing low-temperature-sensitivity can also be disrupted by deleting the entire gene.

The disruption of the gene complementing low-temperature-sensitivity can be carried out, for example, by transforming yeast with a plasmid for the disruption of the gene complementing low-temperature-sensitivity of the yeast or a fragment of the plasmid to induce homologous recombination of a DNA fragment carried on the transforming plasmid or its fragment with the gene on the genome of the yeast. The plasmid for the disruption of the gene complementing low-temperature-sensitivity or its fragment must have homology to the gene complementing low-temperature-sensitivity on the genome of the yeast in a degree sufficient for the induction of homologous recombination. A DNA fragment can be examined for the capability of inducing homologous recombination by introducing the DNA fragment into yeast, and then examining whether a strain carrying homologous recombination, that is, a strain having low-temperature-sensitive



fermentability can be isolated.

Suitable vectors to be used for the construction of the plasmid for the disruption of the gene complementing low-temperature-sensitivity include vectors capable of being maintained in yeast as well as vectors capable of being maintained in Escherichia coli such as pUC19, pBR322, and BluescriptII SK<sup>+</sup>.

As the marker gene, any marker genes which can be used in yeast are usable. Examples of suitable genes are genes complementing auxotrophic mutation such as URA3, TRP1, LEU2, and HIS3, and genes relating to resistance to chemicals such as G418, hygromycin B, cerulenin, and parafluorophenylalanine [e.g. J. Ferment. Bioeng., 76, 60-63 (1993), and Enzyme and Microb. Technol., 15, 874-876 (1993)].

The gene complementing low-temperature-sensitivity on the genome of yeast can be disrupted by transforming the yeast with the plasmid for the disruption of the gene complementing low-temperature-sensitivity.

The transformation of the yeast can be carried out according to the methods generally used in genetic engineering and biological engineering such as the spheroplast method, the lithium acetate method, and the electroporation method mentioned above.

Introduction of the marker gene into the plasmid for the disruption of the gene complementing low-temperature-sensitivity enables ready isolation of a transformant by using the marker as an indicator. The transformant can also be isolated based on the exhibition of low-temperature-sensitive fermentability, which is an indication of the disruption of the gene complementing low-temperature-sensitivity on the genome of the yeast. The low-temperature-sensitivity of the strain of which the gene complementing low-temperature-sensitivity has been disrupted can be confirmed by examining the yeast for the growth or fermentability at a low temperature.

By the above-described process, yeast having low-temperature-sensitive fermentability which is characterized in that the gene complementing low-temperature-sensitivity is inactivated can be obtained. An example of such yeast

5 is Saccharomyces cerevisiae YHK1243 (hereinafter referred to as YHK1243 strain). This strain was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry (1-3, Higashi 1-chome,

10 Tsukuba-shi, Ibaraki-ken) on December 7, 1995 with accession number FERM BP-5327 under the Budapest Treaty.

The following Test Examples show that the low-temperature-sensitivity of fermentability of YHK1243 strain is lowered.

15 Test Example 1 Test on low-temperature-sensitivity of fermentability

One loopful of YHK1243 strain was inoculated into 5 ml of YPD medium comprising 1% yeast extract, 2% peptone and

20 2% glucose in a test tube, and cultured at 30°C for 16 hours. The resulting culture (1 ml) was inoculated into 50 ml of YPD medium in a 300-ml Erlenmeyer flask, and cultured at 30°C for 24 hours. After the completion of culturing, the cells were collected by centrifugation and washed twice

25 with deionized water. The obtained wet cells (0.61 g) were suspended in 50 ml of a fermentation test medium [0.67% Yeast Nitrogen Base w/o Amino Acid (Difco Laboratories Inc.), 2% sucrose, and 1% sodium succinate (adjusted to pH 4.5 with concentrated hydrochloric acid)] in a test tube

30 (inside diameter: 22 mm, height: 200 mm). A silicone stopper equipped with a silicone tube was put in the test tube, and culturing was carried out at 5°C for 24 hours. The gas generated during the culturing was collected in a saturated aqueous solution of sodium chloride via the

35 silicone tube, and the volume of the gas was measured to calculate the amount of carbon dioxide gas generated per gram of yeast cells. The same procedure as above was also

carried out on YOY655 strain to calculate the amount of carbon dioxide gas generated per gram of cells.

The results are shown in Table 1.

5

Table 1

| Strain         | Amount of Carbon Dioxide Gas<br>(ml/g of cells*) |
|----------------|--|
| YOY655 strain  | 133  |
| YHK1243 strain | 15   |

\*: Converted as yeast cells having a dry matter content of 27%

10

The amount of carbon dioxide gas generated by YHK1243 strain at 5°C was approximately 1/9 of that by YOY655 strain.

Test Example 2 Test on low-temperature-sensitivity of fermentability (2)

15

One loopful of YHK1243 strain was inoculated into 30 ml of YPD medium in a 300-ml Erlenmeyer flask, and cultured at 30°C for 24 hours. The whole of the resulting culture was inoculated into 270 ml of a molasses medium (3%

20

molasses, 0.193% urea, 0.046% potassium dihydrogenphosphate, and 2 drops of defoaming agent) in a 2-ℓ Erlenmeyer flask with baffles, and cultured at 30°C for 24 hours. After the completion of culturing, the cells were collected by centrifugation and washed twice with

25

deionized water, followed by dehydration on a clay plate. The same procedure as above was also carried out on YOY655 strain to obtain cells.

30

The obtained cells of YHK1243 strain and YOY655 strain were respectively used for preparing dough according to the following dough composition and steps.

## Dough Composition:

|    |  | (weight: g) |
|----|--|-------------|
|    | Hard flour                                       | 100         |
|    | Sugar  | 5           |
| 5  | Salt   | 2           |
|    | Yeast cells (YHK1243 strain<br>or YOY655 strain) | 3           |
|    | Water  | 62          |
| 10 |  |             |

## Steps:

- Mixing  
(at 100 rpm for 2 minutes with National Complete Mixer)
- ↓
- 15 Dividing  
(the dough is divided into five equal parts;  
34.4 g each)
- ↓
- 20 Storage under refrigeration  
(in a refrigerator at 5°C for 7 days)
- ↓
- Thawing  
(at 30°C and 85% relative humidity for 30  
25 minutes)
- ↓
- Measurement of the amount of carbon dioxide gas  
generated at 30°C in 2 hours with Fermograph (ATTO  
Co., Ltd.)
- 30
- Each dough was stored under refrigeration, and then  
the amount of carbon dioxide gas generated at 30°C was  
measured for evaluation of the refrigeration resistance of  
the dough.
- 35 The results are shown in Table 2.

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Table 2

| Strain         | Amount of Carbon Dioxide Gas (ml)     |                                      |
|----------------|---------------------------------------|--------------------------------------|
|                | Before Storage<br>under Refrigeration | After Storage<br>under Refrigeration |
| YOY655 strain  | 124                                   | 68                                   |
| YHK1243 strain | 120                                   | 101                                  |

The dough containing YHK1243 strain generated a large amount of carbon dioxide gas at 30°C after the storage under refrigeration, compared with the dough containing YOY655 strain. Further, rising of the dough containing YOY655 strain was observed during the storage under refrigeration, whereas rising of the dough containing YHK1243 strain was not substantially observed.

The dough containing the yeast belonging to the genus Saccharomyces and having low-temperature-sensitive fermentability which is characterized in that the gene complementing low-temperature-sensitivity is inactivated (hereinafter referred to as the yeast of the present invention) is described below.

The dough containing the yeast of the present invention refers to the dough prepared by mixing flour or rye flour with the yeast of the present invention, salt, water, and if necessary, additional ingredients such as fats and oils, sugar, shortening, butter, skim milk, yeast food, and eggs, and kneading the mixture.

The refrigeration conditions for storing the dough containing the yeast of the present invention are as follows: at a temperature of -5 to 10°C, preferably 0 to 5°C, for 1 to 10 days, preferably 1 to 7 days.

The process for preparing the dough containing the yeast of the present invention and the process for making bread which comprises adding the yeast of the present invention to dough are described below.

Yeast cells which are suitable for use in bread-making can be obtained by culturing the yeast of the present invention in an ordinary medium containing carbon sources, nitrogen sources, inorganic substances, amino acids, vitamins, etc. at 27 to 32°C under aerobic conditions, collecting the cultured cells, and washing the cells.

Examples of the carbon sources in the medium are glucose, sucrose, starch hydrolyzate, and molasses. Particularly preferred is blackstrap molasses.

Examples of the nitrogen sources are ammonia, ammonium chloride, ammonium sulfate, ammonium carbonate, ammonium acetate, urea, yeast extract, and corn steep liquor.

Examples of the inorganic substances are magnesium phosphate and potassium phosphate. An example of the amino acids is glutamic acid, and examples of the vitamins are pantothenic acid and thiamine.

Fed-batch culture is desirable as the culturing method.

After the completion of culturing, the yeast cells of the present invention are collected by centrifugation or the like. The collected cells are added to flour or rye flour together with salt, water, and if necessary, fats and oils, sugar, shortening, butter, skim milk, yeast food, eggs, etc., followed by mixing, to prepare the dough containing the yeast of the present invention.

Bread can be made according to ordinary methods using the dough obtained as above. There are two kinds of typical methods for making one-loaf bread, buns, etc.; that is, the straight dough method and the sponge-dough method. The former is a method in which all the ingredients are mixed at a time. The latter is a method in which at first a sponge is made by kneading a part of the flour with yeast and water, and then, after fermentation, the remaining ingredients are added to the sponge.

In the straight dough method, all the ingredients are mixed and kneaded, and the kneaded mixture is fermented at 25 to 30°C. The fermented dough is subjected to the

following steps: dividing, benching, molding, proofing (35 to 42°C), and baking (200 to 240°C). In the sponge-dough method, about 70% of the whole flour to be used, yeast, and yeast food are mixed and kneaded with water. The kneaded mixture is fermented at 25 to 35°C for 3 to 5 hours, and then mixed and kneaded with the remaining ingredients such as flour, water, and salt (dough mixing). The obtained dough is subjected to the following steps: dividing, benching, molding, proofing (35 to 42°C), and baking (200 to 240°C).

Danish pastries, croissants, etc. are made, for example, in the following manner.

Flour, salt, the yeast of the present invention, sugar, shortening, eggs, skim milk, and water are mixed and kneaded to prepare dough. Then, fat such as butter or margarine is folded into the dough, and rolling and folding are repeated to make multiple layers of the dough and the fat. This step of folding the fat is called "roll-in", which can be carried out by two methods. In one method, the temperature of the dough to be kneaded is lowered to about 15°C, and the dough is kneaded until the intended number of layers are made without cooling. In the other method, which is the so-called retarding method, cooling is repeated several times using a refrigerator or a freezer in the course of the roll-in step.

The obtained dough is subjected to the following steps: rolling, dividing, molding, proofing (30 to 39°C), and baking (190 to 210°C).

The process for producing ethanol is described below which comprises culturing the yeast of the present invention in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.

The production of ethanol by using the yeast of the present invention is carried out by a conventional method for culturing yeast. The microorganism to be used in the present invention may be immobilized on a gel carrier such as agar, sodium alginate, polyacrylamide, or carageenan.

As the medium for the production of ethanol according to the present invention, either a synthetic medium or a natural medium may be used insofar as it appropriately contains carbon sources, nitrogen sources, inorganic substances, and other nutrients as required.

As the carbon sources, fermentation materials containing at least sucrose should be used. Other carbon sources which can be assimilated by the microorganism used such as sugars (e.g. glucose, fructose, galactose, and maltose) may also be used. As the fermentation materials containing sucrose, any synthetic or natural fermentation materials containing sucrose can be used; examples of suitable materials are sugarcane juice, sugar beet juice, and blackstrap molasses which is obtained after crystallization of sucrose in the process of producing sugar from such juices.

Examples of the nitrogen sources include organic or inorganic nitrogen sources such as urea, ammonia, ammonium sulfate, and ammonium nitrate, and natural nitrogen sources such as corn steep liquor, peptone, meat extract, and yeast extract.

Examples of the inorganic salts are potassium phosphate, sodium phosphate, magnesium sulfate, manganese sulfate, ferrous sulfate, potassium chloride, and sodium chloride.

As the other nutrients, vitamins such as thiamine hydrochloride, p-aminobenzoic acid, folic acid, riboflavin, and inositol, etc. can be used.

Culturing is usually carried out under aerobic conditions, for example, by shaking culture or aeration stirring culture. The culturing temperature is 25 to 50°C, preferably 30 to 43°C, and the pH is maintained at 3 to 7, preferably 4 to 6 during the culturing. Usually, the culturing is completed in 1 to 10 days.

After the completion of culturing, ethanol can be recovered from the culture by ordinary methods such as distillation.



### Brief Description of the Drawings

Fig. 1 shows the restriction map of the DNA fragment containing CSF1 gene and the results of the subcloning and complementation test carried out for the determination of the functional region of CSF1 gene. Fig. 2 illustrates the steps for constructing the plasmid for the disruption of CSF1 gene.

### Best Mode for Carrying Out the Invention

#### 10 Example 1 Cloning of the gene complementing low-temperature-sensitivity

##### (1) Conferment of ura3 mutation on RZT-3 strain

RZT-3 strain, which is a yeast strain having low-temperature-sensitive fermentability, was conferred with ura3 mutation as a marker for introducing a plasmid according to the method of Boeke, et al. [Mol. Gen. Genet., 197, 345-346 (1984)]. That is, one loopful of RZT-3 strain was inoculated into YPD medium and cultured overnight at 30°C with shaking. The resulting culture (100 µl) was smeared on FOA plate [0.67% Yeast Nitrogen Base w/o Amino Acid (Difco Laboratories Inc.), 0.1% 5-fluoroorotic acid, 0.005% uracil, 2% glucose, and 2% agar], and cultured at 30°C for 3 days. From the colonies formed by the culturing was selected a strain having uracil-requirement which is complemented by transformation with plasmid YCp50 carrying URA3 as a marker, and having low-temperature-sensitive fermentability. This strain was designated Saccharomyces cerevisiae RZT-3u (hereinafter referred to as RZT-3u strain).

##### 30 (2) Cloning

The chromosomal DNA of X2180-1B strain (obtained from Yeast Genetic Stock Center) was partially digested with Sau3AI, and the obtained DNA fragments were inserted into the BamHI site of plasmid YCp50 to prepare the gene library. RZT-3u strain was transformed with the gene

library, followed by selection of non-uracil-requiring transformants. The obtained transformants were cultured on YPG agar medium at 30°C to form colonies. Then, a pigment agar was layered over the medium and culturing was carried out at 5°C for 1 to 3 days. A strain showing the color change to yellow around the colony during the culturing at 5°C, that is, a strain of which the fermentation was observed at 5°C, was isolated as a strain of which the mutation exhibiting low-temperature-sensitive fermentability was complemented. From this strain was extracted recombinant plasmid pHK162.

Plasmid pHK162 was introduced into Escherichia coli JM109 strain to prepare Escherichia coli EHK162 strain. The obtained strain was deposited with the National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Ministry of International Trade and Industry on December 7, 1995 with accession number FERM BP-5328 under the Budapest Treaty.

### (3) Complementation test

Plasmid pHK162 carried an inserted Sau3AI/BamHI-BamHI fragment of about 12 Kbp. This plasmid was cleaved with various restriction enzymes and the obtained DNA fragments were separated by electrophoresis, followed by measurement of molecular weights, to prepare the restriction map as shown in Fig. 1. On the basis of this restriction map, recombinant plasmids were constructed by inserting each of the DNA fragments obtained by cleavage of the ca. 12 Kbp Sau3AI/BamHI-BamHI fragment with SphI, BamHI, MluI and ClaI into plasmid YCp50. The recombinant plasmids were used for transforming RZT-3u strain.

The obtained transformants were examined for complementation of the mutation exhibiting low-temperature-sensitive fermentability. As shown in Fig. 1, transformation of RZT-3u strain with plasmid pHK162 resulted in complementation of the mutation exhibiting low-temperature-sensitive fermentability, but transformation of

the strain with the other recombinant plasmids did not complement the mutation exhibiting low-temperature-sensitive fermentability.

The above result shows that a DNA fragment which comprises the DNA fragment of about 6.5 Kbp from BamHI (A) (the sequence at positions 1291 through 1296 in the nucleotide sequence of SEQ ID NO: 1) to SphI (B) (the sequence at positions 7675 through 7680 in the nucleotide sequence of SEQ ID NO: 1) shown in Fig. 1 and additional sequences extending upstream of the 5' end and downstream of the 3' end of the BamHI-SphI fragment is necessary for complementing the mutation exhibiting low-temperature-sensitive fermentability of RZT-3u strain.

#### (4) Determination of nucleotide sequence

The nucleotide sequence of the 12 Kbp DNA fragment inserted into plasmid pHK162 was determined by the dideoxy method using a DNA sequencer (Pharmacia LKB, ALF DNA Sequencer II). As a result, a gene was found which comprises the region of about 6.5 Kbp cleaved at BamHI (A) and SphI (B) shown in Fig. 1 within the open reading frame. This gene was designated CSF1 gene. As shown in the amino acid sequence of SEQ ID NO: 1, the polypeptide encoded by CSF1 gene which is presumed from the determined nucleotide sequence consists of 2958 amino acid residues (molecular weight: 338 kDa). DNA homology search with other genes revealed that the sequence of the upstream region in CSF1 gene comprising about 140 N-terminal amino acid residues in the open reading frame of CSF1 gene coincided with the sequence of the region located upstream of the sequence which was reported as the nucleotide sequence of GAA1 gene of Saccharomyces cerevisiae [Hamburger, et al.: J. Cell Biol., 129, 629-639 (1995)] (the region outside the GAA1 gene-encoding region). However, the report by Hamburger, et al. relates to GAA1 gene and contains no description about the presence of another gene (CSF1 gene) upstream from GAA1 gene. Further, in the nucleotide sequence

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reported by them, one base (T) is inserted between the base at position 198 (T) and the base at position 199 (G) in the nucleotide sequence of SEQ ID NO: 1. Thus, the polypeptide encoded by CSF1 gene cannot be anticipated from the  
5 sequence reported by Hamburger, et al.

Example 2 Preparation of yeast having low-temperature-sensitive fermentability

(1) Construction of plasmid for gene disruption

10 About 5 µg of pHK162 plasmid DNA was dissolved in 20 µl of H buffer [50 mM Tris hydrochloride buffer (pH 7.5), 10 mM magnesium chloride, 1 mM dithiothreitol, and 100 mM sodium chloride], and 10 units of restriction enzyme BamHI was added thereto. Reaction was carried out at 30°C for 3  
15 hours, followed by separation of the reaction product by 0.8% agarose gel electrophoresis. The segment of the gel containing the band of the DNA fragment of about 8 kb from BamHI (A) to BamHI (C) shown in Fig. 1 was cut out, and the fragment was extracted and purified by using GENECLAN II  
20 Kit (Bio 101 Co., Ltd.). The same procedure as above was repeated except that about 5 µg of pUC19 plasmid DNA was used in place of about 5 µg of pHK162 plasmid DNA, whereby a DNA fragment of about 2.8 kb was extracted and purified. The DNA fragment of about 8 kb derived from plasmid pHK162  
25 (1 µg) and the DNA fragment of about 2.8 kb derived from plasmid pUC19 (0.1 µg) were subjected to ligation reaction overnight at 16°C using Ligation Pack (Nippon Gene Co., Ltd.). The reaction mixture (2 µl) was used for transformation of competent high E. coli JM109 strain  
30 (Toyobo Co., Ltd.). The obtained transformant was smeared on 5-bromo-4-chloro-3-indolyl-β-D-galactoside (hereinafter referred to as X-gal) ampicillin LB agar medium and cultured at 37°C for 20 hours. The X-gal ampicillin LB agar medium was prepared by dropping 50 µl of 4% X-gal and  
35 25 µl of isopropyl-1-thio-β-D-galactoside on LB agar medium [1% Bacto-tryptone (Difco Laboratories Inc.), 0.5% yeast

extract, 1% sodium chloride, and 1.5% agar] containing 50 µg/ml ampicillin, and spreading the drops on the medium with a spreader, followed by slight drying. After the completion of culturing, the formed white colony was isolated and cultured. A plasmid DNA was extracted and purified from the culture to obtain plasmid pHK179.

About 5 µg of pHK179 plasmid DNA was dissolved in 20 µl of H buffer, and 10 units each of restriction enzymes MluI and SpeI were added thereto. Reaction was carried out at 37°C for 3 hours. The reaction product was subjected to treatment for making blunt ends by using DNA Blunting Kit (Takara Shuzo Co., Ltd.), followed by separation by 0.8% agarose gel electrophoresis. The segment of the gel containing the band of a fragment of about 10 Kbp excluding the fragment of about 0.6 kb from MluI (the sequence at positions 4388 through 4393 in the nucleotide sequence of SEQ ID NO: 1) to SpeI (the sequence at positions 5027 through 5032 in the nucleotide sequence of SEQ ID NO: 1) shown in Fig. 1 was cut out, and the fragment was extracted and purified by using GENECLAN II Kit. Separately, about 5 µg of YEp24 plasmid DNA, which is a vector carrying the marker gene URA3 complementing uracil-requirement mutation between the HindIII sites, was dissolved in 20 µl of M buffer [10 mM Tris hydrochloride buffer (pH 7.5), 10 mM magnesium chloride, 1 mM dithiothreitol, and 50 mM sodium chloride]. Ten units of restriction enzyme HindIII was added to the solution, and reaction was carried out at 37°C for 3 hours. The reaction product was subjected to treatment for making blunt ends by using DNA Blunting Kit (Takara Shuzo Co., Ltd.), followed by separation by 0.8% agarose gel electrophoresis. The segment of the gel containing the band of a fragment of about 1.1 kb carrying URA3 was cut out, and the fragment was extracted and purified by using GENECLAN II Kit. The DNA fragment of about 10 kb derived from plasmid pHK179 (0.5 µg) and the DNA fragment of about 1.1 kb derived from plasmid YEp24 (0.5 µg) were subjected to ligation reaction overnight at

16°C using Ligation Pack. The reaction mixture (2 µl) was used for transformation of competent high E. coli JM109 strain. The obtained transformant was smeared on LB agar medium containing 50 µg/ml ampicillin and cultured at 37°C for 20 hours. After the completion of culturing, the formed colony was isolated and cultured. A plasmid DNA was extracted and purified from the culture to obtain plasmid pHK188 for disruption of CSF1 gene. Plasmid pHK188 was confirmed to be the desired plasmid by subjecting the plasmid to 0.8% agarose gel electrophoresis and measuring the molecular weight before and after cleavage of the plasmid with BamHI.

The outline of the steps for constructing the plasmid for the disruption of CSF1 gene is shown in Fig. 2.

## (2) Disruption of CSF1 gene

Disruption of CSF1 gene carried by YOY655u strain, which is a monoploid strain of Saccharomyces cerevisiae, was carried out by using plasmid pHK188. YOY655u strain is a strain prepared by introducing uracil-requirement (ura3) mutation into YOY655 strain, which is a monoploid strain of Saccharomyces cerevisiae. The properties such as fermentability of YOY655u strain are the same as those of YOY655 strain. YOY655u strain was inoculated into 100 ml of YPD medium in an Erlenmeyer flask, and cultured with shaking at 30°C until the cell density reached  $2-4 \times 10^7$ . After the completion of culturing, the cells were collected by centrifugation (2500 rpm, 5 minutes) and then brought into contact with plasmid pHK188 by the lithium acetate method. In order to accelerate the homologous recombination of CSF1 gene with plasmid pHK188, plasmid pHK188 had been linearized by complete digestion with BamHI prior to the transformation. YOY655u strain contacted with plasmid pHK188 was inoculated on SGlu agar medium (0.67% Yeast Nitrogen Base w/o Amino Acid, 2% glucose, and 2% agar), and cultured at 30°C for 2 to 5 days. After the

completion of culturing, YHK1243 strain was obtained from one of the formed colonies as a transformant in which the uracil-requirement of YOY655u strain was complemented.

YHK1243 strain, YOY655u strain and RZT-3 strain were inoculated on YPG agar medium, and cultured at 30°C for 1 to 2 days to form colonies. Then, a pigment agar was layered over the medium, followed by culturing at 5°C for 3 days. No color change was observed around the colonies of YHK1243 strain and RZT-3 strain during the culturing, whereas the color around the colony of YOY655u strain changed to yellow on the first day of culturing.

### Example 3 Process for making bread with refrigerated dough

#### (1) Culturing of baker's yeast

YOY655 strain and YHK1243 strain were respectively cultured in the following manner. That is, one loopful of each strain was inoculated into 30 ml of YPD medium in a 300-ml Erlenmeyer flask, and cultured at 30°C for 24 hours. The whole of the resulting culture was inoculated into 270 ml of a molasses medium (3% molasses, 0.193% urea, 0.046% potassium dihydrogenphosphate, and 2 drops of defoaming agent) in a 2-l Erlenmeyer flask with baffles, and cultured at 30°C for 24 hours. After the completion of culturing, the cells were collected by centrifugation and washed twice with deionized water, followed by dehydration on a clay plate. The obtained cells were used for making bread.

#### (2) Preparation of bread

Bread was made according to the following dough composition and steps.

#### Dough Composition:

|            | (weight: g) |
|------------|-------------|
| Hard flour | 100         |
| 35 Sugar   | 5           |
| Salt       | 2           |

|             |    |
|-------------|----|
| Yeast cells | 2  |
| Water       | 62 |

Steps:

- 5      Mixing      (100 rpm, 2 minutes)
- Dividing    (34.4 g)
- Storage     (5°C, 7 days)
- Proofing    (40°C, 90% RH, 75 minutes)
- Baking      (220°C, 25 minutes)

10

The bread obtained using YHK1243 strain as yeast cells had a large volume compared with the bread obtained using YOY655 strain.

15    Example 4    Alcohol fermentation

Culturing of yeast and alcohol fermentation

- YOY655 strain and YHK1243 strain were respectively cultured in the following manner. That is, one loopful of each strain was inoculated into 5 ml of YPD medium in a
- 20    test tube, and cultured at 30°C for 24 hours. After the completion of culturing, 2 ml of the culture was inoculated into 20 ml of a molasses medium (25% molasses and 0.2% ammonium sulfate) in a large test tube, followed by
  - culturing at 37°C. Samples of the culture (0.5 ml each)
  - 25    were taken 16 hours and 40 hours after the start of culturing and analyzed for ethanol concentration.

The results are shown in Table 3.

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Table 3

| Culturing Time | Ethanol production (%) |                |
|----------------|------------------------|----------------|
|                | YOY655 strain          | YHK1243 strain |
| 16 hours       | 4.92*                  | 5.37*          |
| 40 hours       | 10.8*                  | 11.2*          |

\*: The difference was significant at the 5% level of significance.

5

As shown in Table 3, a large amount of ethanol was produced at 37°C by the use of YHK1243 strain compared with YOY655 strain.

#### 10 Industrial Applicability

The present invention provides a protein and a gene which complement the mutation exhibiting low-temperature-sensitive fermentability, refrigeration-resistant yeast which is obtained by inactivation of said gene, and  
15 processes for producing bread and ethanol using said yeast.

09678023 1004000  
004007 2208/950

## Sequence Listing

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(ii) TITLE OF INVENTION: NOVEL YEAST GENES

(iii) NUMBER OF SEQUENCES: 001

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(A) MEDIUM TYPE: Diskette - 3.50 inch, 1440 Kb storage.  
(B) COMPUTER: IBM PS/V  
(C) OPERATING SYSTEM: MS-DOS Ver3.30  
(D) SOFTWARE: PATENT AID Ver1.0

## (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: JP343700/95  
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## (2) INFORMATION FOR SEQ ID NO: 1 :

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8874 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: Genomic DNA

## (vi) ORIGINAL SOURCE:

(A) ORGANISM: *Saccharomyces cerevisiae*

(B) STRAIN: X2180-1B

## (ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1 to 8874

(C) IDENTIFICATION METHOD: E

## (ix) FEATURE:

(A) NAME/KEY: cleavage-site

(B) LOCATION: 1291 to 1296

(C) IDENTIFICATION METHOD: S

## (ix) FEATURE:

(A) NAME/KEY: cleavage-site

(B) LOCATION: 4388 to 4393

(C) IDENTIFICATION METHOD: S

## (ix) FEATURE:

(A) NAME/KEY: cleavage-site

(B) LOCATION: 5927 to 5932

(ix)FEATURE:

(B) LOCATION: 7675 to 7680

(C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| ATG | GAA | GCT | ATT | TCA | CAA | TTA | CGT | GGT | GTT | CCA | TTG | ACA | CAC | CAA | AAG | 48  |
| Met | Glu | Ala | Ile | Ser | Gln | Leu | Arg | Gly | Val | Pro | Leu | Thr | His | Gln | Lys |     |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |     |
| GAC | TTT | AGC | TGG | GTC | TTT | TTA | GTA | GAT | TGG | ATT | CTC | ACG | GTA | GTA | GTA | 96  |
| Asp | Phe | Ser | Trp | Val | Phe | Leu | Val | Asp | Trp | Ile | Leu | Thr | Val | Val | Val |     |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |     |
| TGT | TTG | ACA | ATG | ATA | TTC | TAC | ATG | GGA | AGA | ATC | TAT | GCA | TAC | CTT | GTA | 144 |
| Cys | Leu | Thr | Met | Ile | Phe | Tyr | Met | Gly | Arg | Ile | Tyr | Ala | Tyr | Leu | Val |     |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |
| AGT | TTT | ATA | TTA | GAA | TGG | CTA | CTA | TGG | AAA | CGA | GCG | AAA | ATC | AAG | ATA | 192 |
| Ser | Phe | Ile | Leu | Glu | Trp | Leu | Leu | Trp | Lys | Arg | Ala | Lys | Ile | Lys | Ile |     |
|     | 50  |     |     |     |     | 55  |     |     |     | 60  |     |     |     |     |     |     |
| AAT | GTT | GAG | ACA | CTT | CGT | GTC | TCC | TTA | CTA | GGT | GGT | CGA | ATA | CAT | TTT | 240 |
| Asn | Val | Glu | Thr | Leu | Arg | Val | Ser | Leu | Leu | Gly | Gly | Arg | Ile | His | Phe |     |
| 65  |     |     |     |     | 70  |     |     |     |     | 75  |     |     |     | 80  |     |     |
| AAA | AAC | CTT | TCC | GTA | ATA | CAC | AAA | GAT | TAT | ACA | ATT | TCG | GTA | TTA | GAG | 288 |
| Lys | Asn | Leu | Ser | Val | Ile | His | Lys | Asp | Tyr | Thr | Ile | Ser | Val | Leu | Glu |     |
|     |     |     | 85  |     |     |     |     |     | 90  |     |     |     | 95  |     |     |     |
| GGT | AGT | TTA | ACA | TGG | AAA | TAC | TGG | CTT | TTA | AAT | TGC | AGA | AAA | GCA | GAA | 336 |
| Gly | Ser | Leu | Thr | Trp | Lys | Tyr | Trp | Leu | Leu | Asn | Cys | Arg | Lys | Ala | Glu |     |
|     |     | 100 |     |     |     |     |     | 105 |     |     |     |     | 110 |     |     |     |
| TTG | ATA | GAG | AAT | AAC | AAG | TCT | TCT | TCT | GGC | AAA | AAA | GCA | AAG | CTT | CCC | 384 |
| Leu | Ile | Glu | Asn | Asn | Lys | Ser | Ser | Ser | Gly | Lys | Lys | Ala | Lys | Leu | Pro |     |
|     |     | 115 |     |     |     |     |     | 120 |     |     |     | 125 |     |     |     |     |
| TGT | AAA | ATT | TCC | GTA | GAA | TGT | GAA | GGT | CTA | GAA | ATT | TTT | ATT | TAC | AAC | 432 |
| Cys | Lys | Ile | Ser | Val | Glu | Cys | Glu | Gly | Leu | Glu | Ile | Phe | Ile | Tyr | Asn |     |
|     | 130 |     |     |     |     | 135 |     |     |     |     | 140 |     |     |     |     |     |

AGA ACA GTG GCG TAC GAT AAT GTT ATA AAC TTA CTA TCA AAA GAT GAA 480  
 Arg Thr Val Ala Tyr Asp Asn Val Ile Asn Leu Leu Ser Lys Asp Glu  
 145 150 155 160

CGC GAT AAA TTT GAA AAA TAC CTT AAT GAG CAT TCT TTT CCT GAA CCT 528  
 Arg Asp Lys Phe Glu Lys Tyr Leu Asn Glu His Ser Phe Pro Glu Pro  
 165 170 175

TTT AGC GAT GGA AGT AGT GCT GAT AAA TTA GAT GAA GAT CTA AGC GAA 576  
 Phe Ser Asp Gly Ser Ser Ala Asp Lys Leu Asp Glu Asp Leu Ser Glu  
 180 185 190

TCT GCA TAC ACA ACG AAC TCT GAT GCA TCA ATT GTT AAT GAC AGG GAC 624  
 Ser Ala Tyr Thr Thr Asn Ser Asp Ala Ser Ile Val Asn Asp Arg Asp  
 195 200 205

TAC CAA GAA ACA GAT ATC GGC AAA CAT CCA AAG CTA CTG ATG TTT TTA 672  
 Tyr Gln Glu Thr Asp Ile Gly Lys His Pro Lys Leu Leu Met Phe Leu  
 210 215 220

CCA ATT GAG CTT AAA TTT AGC CGC GGT TCC CTA CTG TTA GGA AAC AAA 720  
 Pro Ile Glu Leu Lys Phe Ser Arg Gly Ser Leu Leu Leu Gly Asn Lys  
 225 230 235 240

TTC ACG CCA TCT GTT ATG ATT CTA AGT TAT GAA AGT GGA AAA GGC ATA 768  
 Phe Thr Pro Ser Val Met Ile Leu Ser Tyr Glu Ser Gly Lys Gly Ile  
 245 250 255

ATA GAT GTT TTA CCT CCA AAA GAG CGA TTA GAT TTA TAC AGA AAT AAA 816  
 Ile Asp Val Leu Pro Pro Lys Glu Arg Leu Asp Leu Tyr Arg Asn Lys  
 260 265 270

ACA CAG ATG GAA TTC AAA AAC TTC GAA ATT TCT ATC AAA CAA AAT ATT 864  
 Thr Gln Met Glu Phe Lys Asn Phe Glu Ile Ser Ile Lys Gln Asn Ile  
 275 280 285

GGT TAC GAT GAT GCT ATT GGA TTG AAG TTT AAA ATA GAT AGA GGG AAA 912  
 Gly Tyr Asp Asp Ala Ile Gly Leu Lys Phe Lys Ile Asp Arg Gly Lys  
 290 295 300

GTG TCA AAG TTA TGG AAA ACG TTT GTA CGA GTC TTT CAG ATA GTA ACC 960  
 Val Ser Lys Leu Trp Lys Thr Phe Val Arg Val Phe Gln Ile Val Thr  
 305 310 315 320

AAG CCT GTT GTA CCG AAA AAG ACT AAA AAA AGC GCA GGC ACA TCA GAT 1008  
 Lys Pro Val Val Pro Lys Lys Thr Lys Lys Ser Ala Gly Thr Ser Asp  
 325 330 335

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GAC AAT TTC TAT CAT AAA TGG AAA GGT TTA TCT CTT TAT AAG GCT TCT 1056  
 Asp Asn Phe Tyr His Lys Trp Lys Gly Leu Ser Leu Tyr Lys Ala Ser  
 340 345 350  
 GCG GGC GAC GCT AAA GCA AGT GAT TTA GAT GAT GTT GAG TTC GAT TTG 1104  
 Ala Gly Asp Ala Lys Ala Ser Asp Leu Asp Asp Val Glu Phe Asp Leu  
 355 360 365  
 ACG AAC CAT GAA TAT GCT AAA TTT ACA TCA ATT TTA AAA TGC CCA AAG 1152  
 Thr Asn His Glu Tyr Ala Lys Phe Thr Ser Ile Leu Lys Cys Pro Lys  
 370 375 380  
 GTC ACA ATT GCA TAT GAC GTG GAT GTT CCG GGC GTT GTG CCA CAT GGT 1200  
 Val Thr Ile Ala Tyr Asp Val Asp Val Pro Gly Val Val Pro His Gly  
 385 390 395 400  
 GCA CAT CCG ACA ATA CCT GAT ATT GAT GGA CCA GAT GTG GGC AAT AAC 1248  
 Ala His Pro Thr Ile Pro Asp Ile Asp Gly Pro Asp Val Gly Asn Asn  
 405 410 415  
 GGA GCA CCT CCA GAT TTT GCT TTA GAT GTT CAA ATT CAC GGA GGA TCC 1296  
 Gly Ala Pro Pro Asp Phe Ala Leu Asp Val Gln Ile His Gly Gly Ser  
 420 425 430  
 ATC TGT TAC GGA CCT TGG GCT CAA AGA CAA GTC AGT CAT CTA CAA AGA 1344  
 Ile Cys Tyr Gly Pro Trp Ala Gln Arg Gln Val Ser His Leu Gln Arg  
 435 440 445  
 GTT CTA TCA CCG GTA GTT TCA AGG ACA GCC AAA CCT ATA AAA AAA CTC 1392  
 Val Leu Ser Pro Val Val Ser Arg Thr Ala Lys Pro Ile Lys Lys Leu  
 450 455 460  
 CCG CCA GGT TCT AGA AGA ATA TAT ACA CTT TTC AGG ATG AAT ATA TCA 1440  
 Pro Pro Gly Ser Arg Arg Ile Tyr Thr Leu Phe Arg Met Asn Ile Ser  
 465 470 475 480  
 ATA ATG GAA GAT ACT ACT TGG CGT ATA CCG ACG AGG GAA AGT AGC AAA 1488  
 Ile Met Glu Asp Thr Thr Trp Arg Ile Pro Thr Arg Glu Ser Ser Lys  
 485 490 495  
 GAC CCC GAA TTT TTG AAA CAC TAC AAA GAA ACT AAT GAA GAA TAT AGG 1536  
 Asp Pro Glu Phe Leu Lys His Tyr Lys Glu Thr Asn Glu Glu Tyr Arg  
 500 505 510  
 CCA TTT GGA TGG ATG GAT CTC CGA TTT TGT AAG GAC ACC TAT GCA AAT 1584  
 Pro Phe Gly Trp Met Asp Leu Arg Phe Cys Lys Asp Thr Tyr Ala Asn  
 515 520 525

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TTC AAT ATA AGT GTT TGT CCT ACA GTG CAA GGT TTT CAG AAT AAT TTC 1632  
 Phe Asn Ile Ser Val Cys Pro Thr Val Gln Gly Phe Gln Asn Asn Phe  
 530 535 540  
 CAT GTT CAT TTC CTG GAA ACC GAA ATT AGG TCA AGT GTT AAT CAC GAT 1680  
 His Val His Phe Leu Glu Thr Glu Ile Arg Ser Ser Val Asn His Asp  
 545 550 555 560  
 ATT TTG TTA AAA AGC AAG GTA TTC GAT ATT GAT GGG GAT ATT GGA TAT 1728  
 Ile Leu Leu Lys Ser Lys Val Phe Asp Ile Asp Gly Asp Ile Gly Tyr  
 565 570 575  
 CCA TTG GGT TGG AAT AGC AAA GCT ATA TGG ATA ATT AAC ATG AAG TCA 1776  
 Pro Leu Gly Trp Asn Ser Lys Ala Ile Trp Ile Ile Asn Met Lys Ser  
 580 585 590  
 GAA CAA TTA GAG GCG TTT CTG CTA CGT GAG CAT ATA ACT TTA GTT GCA 1824  
 Glu Gln Leu Glu Ala Phe Leu Leu Arg Glu His Ile Thr Leu Val Ala  
 595 600 605  
 GAT ACG CTT TCA GAC TTT TCC GCT GGT GAT CCT ACG CCT TAC GAA CTT 1872  
 Asp Thr Leu Ser Asp Phe Ser Ala Gly Asp Pro Thr Pro Tyr Glu Leu  
 610 615 620  
 TTT AGA CCA TTC GTA TAC AAA GTC AAT TGG GAA ATG GAA GGA TAT TCC 1920  
 Phe Arg Pro Phe Val Tyr Lys Val Asn Trp Glu Met Glu Gly Tyr Ser  
 625 630 635 640  
 ATT TAC TTA AAC GTC AAT GAT CAC AAT ATT GTT AAC AAT CCG TTA GAT 1968  
 Ile Tyr Leu Asn Val Asn Asp His Asn Ile Val Asn Asn Pro Leu Asp  
 645 650 655  
 TTT AAC GAA AAC TGT TAT TTA TCC CTT CAT GGT GAT AAG CTT TCA ATT 2016  
 Phe Asn Glu Asn Cys Tyr Leu Ser Leu His Gly Asp Lys Leu Ser Ile  
 660 665 670  
 GAT GTC ACG GTA CCC CGT GAG AGT ATT TTG GGG ACA TAC ACA GAT ATG 2064  
 Asp Val Thr Val Pro Arg Glu Ser Ile Leu Gly Thr Tyr Thr Asp Met  
 675 680 685  
 TCC TAC GAG ATC TCA ACT CCA ATG TTC AGA ATG ATG TTA AAT ACC CCC 2112  
 Ser Tyr Glu Ile Ser Thr Pro Met Phe Arg Met Met Leu Asn Thr Pro  
 690 695 700  
 CCT TGG AAT ACA TTG AAC GAA TTC ATG AAA CAT AAA GAA GTG GGG AGA 2160  
 Pro Trp Asn Thr Leu Asn Glu Phe Met Lys His Lys Glu Val Gly Arg  
 705 710 715 720

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|---|------|
| GCA TAC GAC TTT ACA ATT AAA GGT TCT TAC CTT CTC TAT TCC GAG TTA | 2208 |
| Ala Tyr Asp Phe Thr Ile Lys Gly Ser Tyr Leu Leu Tyr Ser Glu Leu |      |
| 725 730 735   |      |
| GAT ATT GAT AAT GTC GAT ACG CTA GTC ATA GAG TGT AAC AGC AAG AGT | 2256 |
| Asp Ile Asp Asn Val Asp Thr Leu Val Ile Glu Cys Asn Ser Lys Ser |      |
| 740 745 750   |      |
| ACA GTA CTT CAC TGC TAT GGG TTT GTC ATG AGG TAT TTA ACA AAC GTA | 2304 |
| Thr Val Leu His Cys Tyr Gly Phe Val Met Arg Tyr Leu Thr Asn Val |      |
| 755 760 765   |      |
| AAG ATG AAT TAC TTC GGT GAA TTT TTT AAT TTT GTG ACG TCA GAA GAG | 2352 |
| Lys Met Asn Tyr Phe Gly Glu Phe Phe Asn Phe Val Thr Ser Glu Glu |      |
| 770 775 780   |      |
| TAC ACA GGT GTC CTT GGC GCT AGG GAA GTC GGA GAT GTC ACT ACG AAA | 2400 |
| Tyr Thr Gly Val Leu Gly Ala Arg Glu Val Gly Asp Val Thr Thr Lys |      |
| 785 790 795 800   |      |
| AGC TCG GTG GCA GAT TTG GCA TCT ACT GTA GAT TCA GGG TAC CAA AAT | 2448 |
| Ser Ser Val Ala Asp Leu Ala Ser Thr Val Asp Ser Gly Tyr Gln Asn |      |
| 805 810 815   |      |
| AGC AGT CTA AAG AAC GAA TCT GAG GAT AAA GGT CCT ATG AAA AGG TCA | 2496 |
| Ser Ser Leu Lys Asn Glu Ser Glu Asp Lys Gly Pro Met Lys Arg Ser |      |
| 820 825 830   |      |
| GAT TTG AAA AGG ACT ACC AAC GAA ACT GAT ATT TGG TTC ACA TTT TCG | 2544 |
| Asp Leu Lys Arg Thr Thr Asn Glu Thr Asp Ile Trp Phe Thr Phe Ser |      |
| 835 840 845   |      |
| GTT TGG GAT GGT GCT CTG ATA TTA CCA GAA ACG ATT TAC AGT TTT GAT | 2592 |
| Val Trp Asp Gly Ala Leu Ile Leu Pro Glu Thr Ile Tyr Ser Phe Asp |      |
| 850 855 860   |      |
| CCA TGC ATT GCA CTA CAT TTT GCC GAA CTT GTA GTG GAT TTC AGA AGT | 2640 |
| Pro Cys Ile Ala Leu His Phe Ala Glu Leu Val Val Asp Phe Arg Ser |      |
| 865 870 875 880   |      |
| TGT AAT TAT TAT ATG GAC ATA ATG GCG GTT CTC AAC GGG ACT TCA ATA | 2688 |
| Cys Asn Tyr Tyr Met Asp Ile Met Ala Val Leu Asn Gly Thr Ser Ile |      |
| 885 890 895   |      |
| AAG CGG CAC GTT TCA AAA CAA ATA AAT GAA GTA TTT GAT TTT ATA CGT | 2736 |
| Lys Arg His Val Ser Lys Gln Ile Asn Glu Val Phe Asp Phe Ile Arg |      |
| 900 905 910   |      |



CGT AAT AAC GGA GCT GAT GAG CAA GAG CAC GGA TTG CTT TCG GAC CTC 2784  
 Arg Asn Asn Gly Ala Asp Glu Gln Glu His Gly Leu Leu Ser Asp Leu  
 915 920 925

ACC ATT CAT GGA CAT AGA ATG TAT GGA TTA CCA CCC ACA GAA CCT ACC 2832  
 Thr Ile His Gly His Arg Met Tyr Gly Leu Pro Pro Thr Glu Pro Thr  
 930 935 940

TAC TTT TGT CAA TGG GAT ATC AAT CTC GGA GAT TTA TGC ATT GAT TCA 2880  
 Tyr Phe Cys Gln Trp Asp Ile Asn Leu Gly Asp Leu Cys Ile Asp Ser  
 945 950 955 960

GAT ATT GAA TTT ATA AAG GGA TTC TTT AAT TCC TTT TAT AAG ATA GGT 2928  
 Asp Ile Glu Phe Ile Lys Gly Phe Phe Asn Ser Phe Tyr Lys Ile Gly  
 965 970 975

TTT GGC TAC AAT GAC TTG GAA AAT ATA TTA TTA TAT GAC ACT GAG ACC 2976  
 Phe Gly Tyr Asn Asp Leu Glu Asn Ile Leu Leu Tyr Asp Thr Glu Thr  
 980 985 990

ATT AAT GAT ATG ACC TCG CTA ACC GTG CAC GTT GAA AAA ATA AGA ATA 3024  
 Ile Asn Asp Met Thr Ser Leu Thr Val His Val Glu Lys Ile Arg Ile  
 995 1000 1005

GGC CTT AAA GAT CCG GTG ATG AAA TCT CAA TCA GTT ATT AGT GCT GAA 3072  
 Gly Leu Lys Asp Pro Val Met Lys Ser Gln Ser Val Ile Ser Ala Glu  
 1010 1015 1020

TCG ATA TTG TTT ACT TTG ATC GAC TTT GAA AAC GAA AAA TAT TCA CAA 3120  
 Ser Ile Leu Phe Thr Leu Ile Asp Phe Glu Asn Glu Lys Tyr Ser Gln  
 1025 1030 1035 1040

AGA ATA GAC GTG AAA ATT CCA AAA TTG ACA ATT TCG TTA AAT TGC GTG 3168  
 Arg Ile Asp Val Lys Ile Pro Lys Leu Thr Ile Ser Leu Asn Cys Val  
 1045 1050 1055

ATG GGC GAT GGC GTA GAC ACA TCA TTT CTC AAA TTC GAA ACA AAA TTA 3216  
 Met Gly Asp Gly Val Asp Thr Ser Phe Leu Lys Phe Glu Thr Lys Leu  
 1060 1065 1070

AGA TTT ACA AAC TTT GAG CAA TAC AAG GAT ATC GAT AAA AAA AGA TCA 3264  
 Arg Phe Thr Asn Phe Glu Gln Tyr Lys Asp Ile Asp Lys Lys Arg Ser  
 1075 1080 1085

GAA CAA CGC AGA TAT ATA ACA ATA CAC GAT TCA CCC TAT CAT AGG TGT 3312  
 Glu Gln Arg Arg Tyr Ile Thr Ile His Asp Ser Pro Tyr His Arg Cys  
 1090 1095 1100

CCT TTT CTT CTT CCG CTG TTC TAT CAG GAT TCG GAT ACA TAC CAA AAC 3360  
 Pro Phe Leu Leu Pro Leu Phe Tyr Gln Asp Ser Asp Thr Tyr Gln Asn  
 1105 1110 1115 1120  
 CTG TAC GGG GCT ATA GCA CCA TCT TCG TCT ATC CCA ACT TTA CCT CTT 3408  
 Leu Tyr Gly Ala Ile Ala Pro Ser Ser Ser Ile Pro Thr Leu Pro Leu  
 1125 1130 1135  
 CCC ACT TTG CCT GAT ACT ATA GAT TAT ATC ATT GAA GAT ATT GTG GGC 3456  
 Pro Thr Leu Pro Asp Thr Ile Asp Tyr Ile Ile Glu Asp Ile Val Gly  
 1140 1145 1150  
 GAG TAT GCT ACC CTT CTG GAG ACC ACA AAT CCA TTC AAG AAC ATA TTC 3504  
 Glu Tyr Ala Thr Leu Leu Glu Thr Thr Asn Pro Phe Lys Asn Ile Phe  
 1155 1160 1165  
 GCA GAA ACT CCA TCA ACT ATG GAG CCT TCA AGA GCC AGC TTC AGT GAA 3552  
 Ala Glu Thr Pro Ser Thr Met Glu Pro Ser Arg Ala Ser Phe Ser Glu  
 1170 1175 1180  
 GAT GAT AAT GAC GAA GAA GCG GAC CCT TCA AGC TTC AAA CCT GTC GCT 3600  
 Asp Asp Asn Asp Glu Glu Ala Asp Pro Ser Ser Phe Lys Pro Val Ala  
 1185 1190 1195 1200  
 TTT ACA GAA GAC AGA AAC CAC GAA AGG GAT AAC TAT GTT GTT GAT GTT 3648  
 Phe Thr Glu Asp Arg Asn His Glu Arg Asp Asn Tyr Val Val Asp Val  
 1205 1210 1215  
 TCA TAT ATT CTG TTG GAT GTC GAC CCG TTG CTT TTT ATT TTC GCT AAG 3696  
 Ser Tyr Ile Leu Leu Asp Val Asp Pro Leu Leu Phe Ile Phe Ala Lys  
 1220 1225 1230  
 AGT TTA TTA GAA CAG CTT TAC TCT GAA AAC ATG GTA CAA GTC TTA GAC 3744  
 Ser Leu Leu Glu Gln Leu Tyr Ser Glu Asn Met Val Gln Val Leu Asp  
 1235 1240 1245  
 GAT ATT GAA ATT GGG ATT GTG AAA CGA TTA AGC AAC CTT CAA GAA GGG 3792  
 Asp Ile Glu Ile Gly Ile Val Lys Arg Leu Ser Asn Leu Gln Glu Gly  
 1250 1255 1260  
 ATC ACT TCT ATT TCA AAC ATT GAT ATC CAT ATT GCT TAT CTA AAT TTA 3840  
 Ile Thr Ser Ile Ser Asn Ile Asp Ile His Ile Ala Tyr Leu Asn Leu  
 1265 1270 1275 1280  
 ATC TGG CAA GAG ACA GGT GAG GAA GGT TTT GAG CTC TAT TTA GAT CGT 3888  
 Ile Trp Gln Glu Thr Gly Glu Glu Gly Phe Glu Leu Tyr Leu Asp Arg  
 1285 1290 1295

ATT GAT TAT CAA ATG AGT GAA AAG TCT CTA GAG AAG AAC CGA ACA AAT 3936  
 Ile Asp Tyr Gln Met Ser Glu Lys Ser Leu Glu Lys Asn Arg Thr Asn  
 1300 1305 1310  
 AAA TTA TTA GAA GTA GCA GCT TTA GCA AAG GTA AAA ACT GTC AGA GTG 3984  
 Lys Leu Leu Glu Val Ala Ala Leu Ala Lys Val Lys Thr Val Arg Val  
 1315 1320 1325  
 ACT GTT AAC CAG AAG AAA AAT CCA GAC TTG TCT GAA GAT CGT CCC CCT 4032  
 Thr Val Asn Gln Lys Lys Asn Pro Asp Leu Ser Glu Asp Arg Pro Pro  
 1330 1335 1340  
 GCA CTG TCG CTA GGG ATT GAG GGT TTC GAA GTA TGG TCT TCT ACA GAA 4080  
 Ala Leu Ser Leu Gly Ile Glu Gly Phe Glu Val Trp Ser Ser Thr Glu  
 1345 1350 1355 1360  
 GAT AGA CAA GTT AAC TCA TTA AAC TTA ACG TCA TCA GAT ATT ACC ATA 4128  
 Asp Arg Gln Val Asn Ser Leu Asn Leu Thr Ser Ser Asp Ile Thr Ile  
 1365 1370 1375  
 GAC GAA TCT CAA ATG GAA TGG CTG TTT GAG TAC TGT AGT GAC CAG GGA 4176  
 Asp Glu Ser Gln Met Glu Trp Leu Phe Glu Tyr Cys Ser Asp Gln Gly  
 1380 1385 1390  
 AAT CTT ATT CAA GAG GTT TGC ACT TCT TTT AAT TCT ATT CAG AAC ACC 4224  
 Asn Leu Ile Gln Glu Val Cys Thr Ser Phe Asn Ser Ile Gln Asn Thr  
 1395 1400 1405  
 AGA AGT AAT TCA AAG ACA GAA CTC ATT TCA AAG CTC ACA GCC GCA AGC 4272  
 Arg Ser Asn Ser Lys Thr Glu Leu Ile Ser Lys Leu Thr Ala Ala Ser  
 1410 1415 1420  
 GAA TAT TAT CAA ATT AGT CAT GAT CCT TAC GTC ATA ACA AAA CCT GCT 4320  
 Glu Tyr Tyr Gln Ile Ser His Asp Pro Tyr Val Ile Thr Lys Pro Ala  
 1425 1430 1435 1440  
 TTT ATT ATG AGA CTT TCC AAA GGG CAT GTG CGT GAG AAT CGT AGT TGG 4368  
 Phe Ile Met Arg Leu Ser Lys Gly His Val Arg Glu Asn Arg Ser Trp  
 1445 1450 1455  
 AAA ATT ATT ACG CGT CTG AGA CAC ATT TTA ACG TAC CTT CCT GAT GAT 4416  
 Lys Ile Ile Thr Arg Leu Arg His Ile Leu Thr Tyr Leu Pro Asp Asp  
 1460 1465 1470  
 TGG CAA AGC AAC ATC GAC GAA GTG CTA AAA GAA AAG AAA TAT ACC TCT 4464  
 Trp Gln Ser Asn Ile Asp Glu Val Leu Lys Glu Lys Lys Tyr Thr Ser  
 1475 1480 1485

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GCT AAA GAT GCA AAA AAT ATC TTC ATG TCT GTG TTT TCG ACT TGG AGA 4512  
 Ala Lys Asp Ala Lys Asn Ile Phe Met Ser Val Phe Ser Thr Trp Arg  
 1490 1495 1500  
 AAT TGG GAG TTC TCA GAT GTT GCA AGG TCG TAT ATA TAC GGC AAA TTA 4560  
 Asn Trp Glu Phe Ser Asp Val Ala Arg Ser Tyr Ile Tyr Gly Lys Leu  
 1505 1510 1515 1520  
 TTC ACG GCA GAA AAT GAG AAA CAT AAA CAA AAT TTG ATT AAA AAA TTG 4608  
 Phe Thr Ala Glu Asn Glu Lys His Lys Gln Asn Leu Ile Lys Lys Leu  
 1525 1530 1535  
 TTG AAG TGT ACC ATG GGA TCA TTT TAC CTT ACT GTT TAT GGT GAG GGA 4656  
 Leu Lys Cys Thr Met Gly Ser Phe Tyr Leu Thr Val Tyr Gly Glu Gly  
 1540 1545 1550  
 TAT GAG GTT GAG CAT AAT TTT GTT GTT GCG GAT GCC AAT CTG GTA GTG 4704  
 Tyr Glu Val Glu His Asn Phe Val Val Ala Asp Ala Asn Leu Val Val  
 1555 1560 1565  
 GAT TTG ACG CCT CCG GTG ACA AGC TTA CCT TCA AAT CGA GAA GAA ACT 4752  
 Asp Leu Thr Pro Pro Val Thr Ser Leu Pro Ser Asn Arg Glu Glu Thr  
 1570 1575 1580  
 ATT GAA ATT ACG GGA AGA GTA GGC TCA GTA AAA GGA AAA TTC AGT GAT 4800  
 Ile Glu Ile Thr Gly Arg Val Gly Ser Val Lys Gly Lys Phe Ser Asp  
 1585 1590 1595 1600  
 AGG TTA CTT AAA TTG CAA GAT CTT ATT CCA CTC ATT GCA GCA GTG GGC 4848  
 Arg Leu Leu Lys Leu Gln Asp Leu Ile Pro Leu Ile Ala Ala Val Gly  
 1605 1610 1615  
 GAA GAT GAC AAA AGT GAT CCA AAA AAG GAG TTA TCA AAG CAA TTC AAA 4896  
 Glu Asp Asp Lys Ser Asp Pro Lys Lys Glu Leu Ser Lys Gln Phe Lys  
 1620 1625 1630  
 ATG AAC ACC GTT TTA TTA GTG GAT AAA AGT GAA CTG CAA CTG GTC ATG 4944  
 Met Asn Thr Val Leu Leu Val Asp Lys Ser Glu Leu Gln Leu Val Met  
 1635 1640 1645  
 GAC CAA ACG AAG CTG ATG AGT AGA ACA GTT GGG GGT AGA GTT AGT TTA 4992  
 Asp Gln Thr Lys Leu Met Ser Arg Thr Val Gly Gly Arg Val Ser Leu  
 1650 1655 1660  
 CTA TGG GAA AAT CTA AAA GAT TCA ACT AGT CAA GCG GGT TCA TTG GTT 5040  
 Leu Trp Glu Asn Leu Lys Asp Ser Thr Ser Gln Ala Gly Ser Leu Val  
 1665 1670 1675 1680

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ATA TTT TCC CAG AAA TCG GAA GTG TGG TTA AAA CAC ACA TCT GTC ATT 5088  
 Ile Phe Ser Gln Lys Ser Glu Val Trp Leu Lys His Thr Ser Val Ile  
 1685 1690 1695  
 TTG GGA GAA GCT CAA CTG CGC GAC TTT TCA GTT TTA GCG ACT ACT GAG 5136  
 Leu Gly Glu Ala Gln Leu Arg Asp Phe Ser Val Leu Ala Thr Thr Glu  
 1700 1705 1710  
 GCA TGG TCA CAC AAG CCT ACG ATT CTG ATA AAC AAC CAG TGC GCA GAT 5184  
 Ala Trp Ser His Lys Pro Thr Ile Leu Ile Asn Asn Gln Cys Ala Asp  
 1715 1720 1725  
 CTT CAT TTT AGA GCA ATG AGT TCA ACT GAG CAA TTA GTA ACC GCT ATT 5232  
 Leu His Phe Arg Ala Met Ser Ser Thr Glu Gln Leu Val Thr Ala Ile  
 1730 1735 1740  
 ACT GAA ATT AGG GAA AGT CTG ATG ATG ATT AAA GAG CGC ATA AAG TTT 5280  
 Thr Glu Ile Arg Glu Ser Leu Met Met Ile Lys Glu Arg Ile Lys Phe  
 1745 1750 1755 1760  
 AAA CCT AAA TCA AAG AAA AAG TCC CAA TTT GTC GAC CAG AAA ATT AAT 5328  
 Lys Pro Lys Ser Lys Lys Lys Ser Gln Phe Val Asp Gln Lys Ile Asn  
 1765 1770 1775  
 ACA GTC TTG TCA TGT TAT TTT TCA AAC GTT AGT TCT GAA GTT ATG CCG 5376  
 Thr Val Leu Ser Cys Tyr Phe Ser Asn Val Ser Ser Glu Val Met Pro  
 1780 1785 1790  
 CTC TCG CCA TTT TAT ATT CGT CAC GAA GCC AAG CAG CTT GAT ATA TAT 5424  
 Leu Ser Pro Phe Tyr Ile Arg His Glu Ala Lys Gln Leu Asp Ile Tyr  
 1795 1800 1805  
 TTT AAC AAA TTC GGT TCA AAT GAG ATT TTG TTA AGC ATA TGG GAT ACT 5472  
 Phe Asn Lys Phe Gly Ser Asn Glu Ile Leu Leu Ser Ile Trp Asp Thr  
 1810 1815 1820  
 GAT TTT TTC ATG ACA TCG CAC CAG ACA AAG GAG CAA TAC CTA AGG TTT 5520  
 Asp Phe Phe Met Thr Ser His Gln Thr Lys Glu Gln Tyr Leu Arg Phe  
 1825 1830 1835 1840  
 TCA TTT GGC GAT ATT GAA ATT AAA GGA GGA ATT TCT AGA GAA GGC TAT 5568  
 Ser Phe Gly Asp Ile Glu Ile Lys Gly Gly Ile Ser Arg Glu Gly Tyr  
 1845 1850 1855  
 TCG TTG ATA AAC GTT GAC ATC TCA ATA TCT ATG ATT AAG TTA ACC TTT 5616  
 Ser Leu Ile Asn Val Asp Ile Ser Ile Ser Met Ile Lys Leu Thr Phe  
 1860 1865 1870

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TCG GAG CCG CGC CGT ATT GTA AAC AGT TTT TTA CAA GAT GAA AAG CTT 5664  
 Ser Glu Pro Arg Arg Ile Val Asn Ser Phe Leu Gln Asp Glu Lys Leu  
 1875 1880 1885

GCT TCT CAG GGT ATC AAT CTG TTA TAT TCC CTG AAG CCT TTA TTC TTT 5712  
 Ala Ser Gln Gly Ile Asn Leu Leu Tyr Ser Leu Lys Pro Leu Phe Phe  
 1890 1895 1900

AGT TCA AAT CTA CCA AAA AAA GAG AAG CAG GCA CCC TCG ATA ATG ATA 5760  
 Ser Ser Asn Leu Pro Lys Lys Glu Lys Gln Ala Pro Ser Ile Met Ile  
 1905 1910 1915 1920

AAT TGG ACA TTA GAT ACT AGC ATT ACT TAT TTT GGT GTT CTT GTG CCA 5808  
 Asn Trp Thr Leu Asp Thr Ser Ile Thr Tyr Phe Gly Val Leu Val Pro  
 1925 1930 1935

GTG GCT TCC ACG TAT TTC GTG TTT GAA TTA CAT ATG CTG CTA CTT TCT 5856  
 Val Ala Ser Thr Tyr Phe Val Phe Glu Leu His Met Leu Leu Leu Ser  
 1940 1945 1950

CTG ACC AAT ACG AAT AAC GGT ATG TTA CCA GAA GAA ACC AAG GTG ACG 5904  
 Leu Thr Asn Thr Asn Asn Gly Met Leu Pro Glu Glu Thr Lys Val Thr  
 1955 1960 1965

GGA CAG TTT TCC ATC GAA AAC ATC CTA TTT CTA ATA AAG GAG CGG TCA 5952  
 Gly Gln Phe Ser Ile Glu Asn Ile Leu Phe Leu Ile Lys Glu Arg Ser  
 1970 1975 1980

CTA CCC ATT GGT CTT TCC AAA TTA CTC GAC TTT TCC ATA AAA GTA TCA 6000  
 Leu Pro Ile Gly Leu Ser Lys Leu Leu Asp Phe Ser Ile Lys Val Ser  
 1985 1990 1995 2000

ACC CTA CAA AGA ACG GTT GAT ACG GAG CAG TCA TTC CAA GTG GAA AGT 6048  
 Thr Leu Gln Arg Thr Val Asp Thr Glu Gln Ser Phe Gln Val Glu Ser  
 2005 2010 2015

TCT CAT TTC AGG GTC TGC TTA TCT CCT GAT TCT CTA TTA AGA TTA ATG 6096  
 Ser His Phe Arg Val Cys Leu Ser Pro Asp Ser Leu Leu Arg Leu Met  
 2020 2025 2030

TGG GGC GCG CAT AAA TTG CTA GAC TTG AGC CAT TAC TAT TCA AGA CGC 6144  
 Trp Gly Ala His Lys Leu Leu Asp Leu Ser His Tyr Tyr Ser Arg Arg  
 2035 2040 2045

CAT GCC CCT AAT ATT TGG AAC ACT AAG ATG TTC ACC GGT AAA AGT GAT 6192  
 His Ala Pro Asn Ile Trp Asn Thr Lys Met Phe Thr Gly Lys Ser Asp  
 2050 2055 2060

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|---|------|
| AAG TCA AAA GAA ATG CCC ATA AAT TTC CGT TCA ATA CAC ATC CTG TCC | 6240 |
| Lys Ser Lys Glu Met Pro Ile Asn Phe Arg Ser Ile His Ile Leu Ser |      |
| 2065  | 2070 |
| TAT AAA TTT TGT ATT GGG TGG ATA TTC CAG TAT GGA GCA GGC TCC AAT | 6288 |
| Tyr Lys Phe Cys Ile Gly Trp Ile Phe Gln Tyr Gly Ala Gly Ser Asn |      |
| 2085  | 2090 |
| CCT GGG TTA ATG TTA GGT TAT AAC AGA TTG TTT TCA GCA TAT GAA AAG | 6336 |
| Pro Gly Leu Met Leu Gly Tyr Asn Arg Leu Phe Ser Ala Tyr Glu Lys |      |
| 2100  | 2105 |
| GAT TTT GGG AAA TTC ACA GTT GTG GAC GCT TTT TTC TCT GTT GCG AAT | 6384 |
| Asp Phe Gly Lys Phe Thr Val Val Asp Ala Phe Phe Ser Val Ala Asn |      |
| 2115  | 2120 |
| GGT AAT ACC TCA AGC ACT TTT TTC TCT GAA GGA AAC GAG AAA GAC AAA | 6432 |
| Gly Asn Thr Ser Ser Thr Phe Phe Ser Glu Gly Asn Glu Lys Asp Lys |      |
| 2130  | 2135 |
| TAT AAT AGA AGT TTC TTG CCA AAC ATG CAA ATA TCC TAC TGG TTC AAA | 6480 |
| Tyr Asn Arg Ser Phe Leu Pro Asn Met Gln Ile Ser Tyr Trp Phe Lys |      |
| 2145  | 2150 |
| AGA TGT GGT GAG TTG AAA GAT TGG TTT TTT AGA TTT CAT GGT GAA GCA | 6528 |
| Arg Cys Gly Glu Leu Lys Asp Trp Phe Phe Arg Phe His Gly Glu Ala |      |
| 2165  | 2170 |
| CTG GAT GTA AAC TTT GTC CCG TCA TTC ATG GAT GTC ATT GAG TCT ACT | 6576 |
| Leu Asp Val Asn Phe Val Pro Ser Phe Met Asp Val Ile Glu Ser Thr |      |
| 2180  | 2185 |
| TTA CAA TCC ATG CGA GCA TTT CAA GAG CTG AAA AAG AAC ATT CTG GAT | 6624 |
| Leu Gln Ser Met Arg Ala Phe Gln Glu Leu Lys Lys Asn Ile Leu Asp |      |
| 2195  | 2200 |
| GTG TCC GAG AGT TTG CGT GCG GAA AAT GAT AAT TCT TAT GCT AGT ACC | 6672 |
| Val Ser Glu Ser Leu Arg Ala Glu Asn Asp Asn Ser Tyr Ala Ser Thr |      |
| 2210  | 2215 |
| AGT GTC GAA AGT GCT TCG AGT AGT TTG GCT CCC TTT CTC GAT AAC ATT | 6720 |
| Ser Val Glu Ser Ala Ser Ser Ser Leu Ala Pro Phe Leu Asp Asn Ile |      |
| 2225  | 2230 |
| AGA TCT GTT AAC TCA AAT TTC AAG TAT GAC GGT GGT GTA TTT AGG GTT | 6768 |
| Arg Ser Val Asn Ser Asn Phe Lys Tyr Asp Gly Gly Val Phe Arg Val |      |
| 2245  | 2250 |

|   |      |
|---|------|
| TAC ACG TAC GAA GAT ATT GAA ACC AAG AGT GAG CCA TCT TTT GAA ATA | 6816 |
| Tyr Thr Tyr Glu Asp Ile Glu Thr Lys Ser Glu Pro Ser Phe Glu Ile |      |
| 2260 2265 2270  |      |
| AAA AGT CCA GTA GTC ACT ATA AAC TGT ACA TAT AAA CAT GAT GAA GAT | 6864 |
| Lys Ser Pro Val Val Thr Ile Asn Cys Thr Tyr Lys His Asp Glu Asp |      |
| 2275 2280 2285  |      |
| AAA GTT AAG CCA CAT AAA TTC AGA ACA TTA ATC ACT GTC GAC CCA ACG | 6912 |
| Lys Val Lys Pro His Lys Phe Arg Thr Leu Ile Thr Val Asp Pro Thr |      |
| 2290 2295 2300  |      |
| CAT AAT ACT TTG TAT GCG GGA TGT GCT CCT TTA TTA ATG GAA TTT TCT | 6960 |
| His Asn Thr Leu Tyr Ala Gly Cys Ala Pro Leu Leu Met Glu Phe Ser |      |
| 2305 2310 2315 2320   |      |
| GAA AGT CTG CAA AAG ATG ATA AAG AAA CAT AGC ACC GAC GAA AAA CCA | 7008 |
| Glu Ser Leu Gln Lys Met Ile Lys Lys His Ser Thr Asp Glu Lys Pro |      |
| 2325 2330 2335  |      |
| AAC TTT ACA AAA CCT TCT TCA CAG AAT GTT GAT TAT AAG CGA CTT TTG | 7056 |
| Asn Phe Thr Lys Pro Ser Ser Gln Asn Val Asp Tyr Lys Arg Leu Leu |      |
| 2340 2345 2350  |      |
| GAT CAA TTT GAT GTG GCT GTA AAA CTA ACA TCA GCC AAG CAA CAG CTA | 7104 |
| Asp Gln Phe Asp Val Ala Val Lys Leu Thr Ser Ala Lys Gln Gln Leu |      |
| 2355 2360 2365  |      |
| AGT TTG AGC TGT GAA CCA AAA GCT AAG GTT CAG GCA GAT GTT GGA TTT | 7152 |
| Ser Leu Ser Cys Glu Pro Lys Ala Lys Val Gln Ala Asp Val Gly Phe |      |
| 2370 2375 2380  |      |
| GAA TCG TTT TTG TTC AGT ATG GCT ACC AAT GAG TTC GAC TCT GAA CAG | 7200 |
| Glu Ser Phe Leu Phe Ser Met Ala Thr Asn Glu Phe Asp Ser Glu Gln |      |
| 2385 2390 2395 2400   |      |
| CCT TTG GAG TTT TCT TTA ACT CTA GAA CAC ACA AAA GCG TCC ATT AAG | 7248 |
| Pro Leu Glu Phe Ser Leu Thr Leu Glu His Thr Lys Ala Ser Ile Lys |      |
| 2405 2410 2415  |      |
| CAC ATA TTT TCA AGA GAA GTA AGT ACG TCC TTT GAA GTT GGT TTC ATG | 7296 |
| His Ile Phe Ser Arg Glu Val Ser Thr Ser Phe Glu Val Gly Phe Met |      |
| 2420 2425 2430  |      |
| GAC TTG ACG CTT TTA TTT ACA CAT CCT GAT GTA ATC AGT ATG TAT GGA | 7344 |
| Asp Leu Thr Leu Leu Phe Thr His Pro Asp Val Ile Ser Met Tyr Gly |      |
| 2435 2440 2445  |      |



ACG GGG TTG GTT TCT GAT CTA AGC GTC TTC TTC AAT GTA AAG CAG CTC 7392  
 Thr Gly Leu Val Ser Asp Leu Ser Val Phe Phe Asn Val Lys Gln Leu  
 2450 2455 2460  
 CAG AAC CTG TAT TTA TTC TTG GAC ATA TGG AGG TTC AGT AGC ATT TTA 7440  
 Gln Asn Leu Tyr Leu Phe Leu Asp Ile Trp Arg Phe Ser Ser Ile Leu  
 2465 2470 2475 2480  
 CAC ACA CGG CCA GTG CAA AGA ACT GTT AAT AAG GAA ATT GAA ATG AGT 7488  
 His Thr Arg Pro Val Gln Arg Thr Val Asn Lys Glu Ile Glu Met Ser  
 2485 2490 2495  
 TCA TTA ACA TCA ACC AAC TAT GCC GAT GCA GGT ACG GAA ATA CCC TGG 7536  
 Ser Leu Thr Ser Thr Asn Tyr Ala Asp Ala Gly Thr Glu Ile Pro Trp  
 2500 2505 2510  
 TGC TTT ACA TTA ATT TTT ACA AAT GTT AGC GGA GAC GTT GAT TTG GGT 7584  
 Cys Phe Thr Leu Ile Phe Thr Asn Val Ser Gly Asp Val Asp Leu Gly  
 2515 2520 2525  
 CCT TCT CTC GGT ATG ATT TCA TTA AGG ACA CAA AGA ACA TGG CTG GCC 7632  
 Pro Ser Leu Gly Met Ile Ser Leu Arg Thr Gln Arg Thr Trp Leu Ala  
 2530 2535 2540  
 ACA GAT CAT TAT AAC GAG AAG CGG CAG TTA CTG CAT GCT TTC ACT GAC 7680  
 Thr Asp His Tyr Asn Glu Lys Arg Gln Leu Leu His Ala Phe Thr Asp  
 2545 2550 2555 2560  
 GGT ATT AGC TTG ACA TCA GAA GGT AGA CTG AGT GGT TTA TTT GAA GTT 7728  
 Gly Ile Ser Leu Thr Ser Glu Gly Arg Leu Ser Gly Leu Phe Glu Val  
 2565 2570 2575  
 GCG AAT GCA AGT TGG TTA TCA GAA GTA AAA TGG CCA CCT GAA AAA AGC 7776  
 Ala Asn Ala Ser Trp Leu Ser Glu Val Lys Trp Pro Pro Glu Lys Ser  
 2580 2585 2590  
 AAA AAT ACT CAT CCA TTA GTT TCC ACC TCC CTG AAT ATT GAT GAT ATA 7824  
 Lys Asn Thr His Pro Leu Val Ser Thr Ser Leu Asn Ile Asp Asp Ile  
 2595 2600 2605  
 GCG GTA AAG GCT GCT TTT GAT TAT CAT ATG TTC TTA ATC GGC ACT ATA 7872  
 Ala Val Lys Ala Ala Phe Asp Tyr His Met Phe Leu Ile Gly Thr Ile  
 2610 2615 2620  
 AGT AAC ATA CAC TTC CAT CTT CAT AAT GAA AAG GAT GCC AAG GGG GTT 7920  
 Ser Asn Ile His Phe His Leu His Asn Glu Lys Asp Ala Lys Gly Val  
 2625 2630 2635 2640

09678023 100400

|   |      |
|---|------|
| CTA CCT GAT TTG CTG CAG GTC TCT TTT TCA TCA GAT GAA ATT ATC CTC | 7968 |
| Leu Pro Asp Leu Leu Gln Val Ser Phe Ser Ser Asp Glu Ile Ile Leu |      |
| 2645 2650 2655  |      |
| AGC TCT ACT GCA TTA GTT GTA GCA AAT ATA CTG GAT ATC TAC AAC ACC | 8016 |
| Ser Ser Thr Ala Leu Val Val Ala Asn Ile Leu Asp Ile Tyr Asn Thr |      |
| 2660 2665 2670  |      |
| ATT GTA CGT ATG AGG CAG GAT AAT AAA ATA TCG TAT ATG GAG ACG TTG | 8064 |
| Ile Val Arg Met Arg Gln Asp Asn Lys Ile Ser Tyr Met Glu Thr Leu |      |
| 2675 2680 2685  |      |
| AGA GAT TCC AAT CCT GGT GAA TCT AGG CAA CCA ATA TTA TAC AAA GAC | 8112 |
| Arg Asp Ser Asn Pro Gly Glu Ser Arg Gln Pro Ile Leu Tyr Lys Asp |      |
| 2690 2695 2700  |      |
| ATT TTA AGA TCG CTG AAA TTA CTC AGA ACT GAT CTC TCG GTG AAT ATC | 8160 |
| Ile Leu Arg Ser Leu Lys Leu Leu Arg Thr Asp Leu Ser Val Asn Ile |      |
| 2705 2710 2715 2720   |      |
| TCC TCT TCA AAG GTC CAG ATT TCG CCA ATA TCT TTA TTC GAT GTG GAA | 8208 |
| Ser Ser Ser Lys Val Gln Ile Ser Pro Ile Ser Leu Phe Asp Val Glu |      |
| 2725 2730 2735  |      |
| GTG TTA GTA ATA AGA ATT GAC AAA GTC TCT ATA CGT TCC GAA ACA CAT | 8256 |
| Val Leu Val Ile Arg Ile Asp Lys Val Ser Ile Arg Ser Glu Thr His |      |
| 2740 2745 2750  |      |
| TCG GGG AAA AAA TTA AAG ACA GAT TTG CAA CTA CAA GTT TTA GAT GTT | 8304 |
| Ser Gly Lys Lys Leu Lys Thr Asp Leu Gln Leu Gln Val Leu Asp Val |      |
| 2755 2760 2765  |      |
| TCT GCA GCG CTT TCT ACT TCC AAA GAA GAA TTA GAT GAG GAA GTT GGA | 8352 |
| Ser Ala Ala Leu Ser Thr Ser Lys Glu Glu Leu Asp Glu Glu Val Gly |      |
| 2770 2775 2780  |      |
| GCT TCC ATT GCT ATT GAT GAT TAC ATG CAT TAT GCT TCC AAG ATT GTC | 8400 |
| Ala Ser Ile Ala Ile Asp Asp Tyr Met His Tyr Ala Ser Lys Ile Val |      |
| 2785 2790 2795 2800   |      |
| GGT GGT ACT ATC ATT GAT ATT CCA AAA CTT GCT GTT CAT ATG ACA ACT | 8448 |
| Gly Gly Thr Ile Ile Asp Ile Pro Lys Leu Ala Val His Met Thr Thr |      |
| 2805 2810 2815  |      |
| TTA CAA GAA GAA AAG ACA AAT AAT TTA GAA TAT CTA TTT GCT TGC TCT | 8496 |
| Leu Gln Glu Glu Lys Thr Asn Asn Leu Glu Tyr Leu Phe Ala Cys Ser |      |
| 2820 2825 2830  |      |

09578023 100400

TTT TCA GAC AAA ATA TCT GTA AGG TGG AAT CTA GGG CCT GTA GAC TTC 8544  
 Phe Ser Asp Lys Ile Ser Val Arg Trp Asn Leu Gly Pro Val Asp Phe  
 2835 2840 2845  
 ATA AAG GAA ATG TGG ACT ACA CAT GTC AAA GCA CTG GCA GTT CGT CGA 8592  
 Ile Lys Glu Met Trp Thr Thr His Val Lys Ala Leu Ala Val Arg Arg  
 2850 2855 2860  
 TCC CAG GTA GCA AAT ATT TCC TTT GGA CAA ACT GAG GAA GAA CTT GAA 8640  
 Ser Gln Val Ala Asn Ile Ser Phe Gly Gln Thr Glu Glu Glu Leu Glu  
 2865 2870 2875 2880  
 GAA TCA ATT AAA AAG GAA GAA GCC GCT TCA AAG TTT AAT TAT ATT GCA 8688  
 Glu Ser Ile Lys Lys Glu Glu Ala Ala Ser Lys Phe Asn Tyr Ile Ala  
 2885 2890 2895  
 CTA GAA GAA CCG CAG ATC GAA GTG CCT CAG ATA AGA GAT CTG GGA GAC 8736  
 Leu Glu Glu Pro Gln Ile Glu Val Pro Gln Ile Arg Asp Leu Gly Asp  
 2900 2905 2910  
 GCC ACT CCA CCT ATG GAA TGG TTT GGT GTC AAT AGA AAA AAA TTT CCG 8784  
 Ala Thr Pro Pro Met Glu Trp Phe Gly Val Asn Arg Lys Lys Phe Pro  
 2915 2920 2925  
 AAA TTC ACT CAC CAA ACC GCA GTT ATC CCC GTC CAA AAG CTT GTT TAT 8832  
 Lys Phe Thr His Gln Thr Ala Val Ile Pro Val Gln Lys Leu Val Tyr  
 2930 2935 2940  
 CTT GCA GAA AAG CAG TAT GTC AAG ATA CTA GAT GAT ACG CAT 8874  
 Leu Ala Glu Lys Gln Tyr Val Lys Ile Leu Asp Asp Thr His  
 2945 2950 2955

004001" E2084960

## CLAIMS

1. A gene which encodes a protein having the amino acid sequence represented by SEQ ID NO: 1, or encodes a protein being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence represented by SEQ ID NO: 1.

2. A gene which comprises DNA having the nucleotide sequence represented by SEQ ID NO: 1, or comprises DNA being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having a nucleotide sequence wherein one or more DNAs are added, deleted or substituted in the nucleotide sequence represented by SEQ ID NO: 1.

3. A protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence represented by SEQ ID NO: 1.

4. Yeast belonging to the genus Saccharomyces and having low-temperature-sensitive fermentability which is characterized in that the gene according to Claim 1 or 2 on the chromosome is inactivated.

5. The yeast according to Claim 4, wherein the yeast belongs to Saccharomyces cerevisiae.

6. The yeast according to Claim 4 or 5, wherein the sequence at positions 4388 through 7885 in the nucleotide sequence represented by SEQ ID NO: 1 is disrupted.

7. Saccharomyces cerevisiae YHK1243 (FERM BP-5327).

10. A process for producing ethanol which comprises culturing the yeast according to any of Claims 4-7 in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.

## ABSTRACT

00400T" E203/960

5 The present invention relates to a protein having the amino acid sequence represented by SEQ ID NO: 1, or a protein being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having an amino acid sequence wherein one or more amino acid residues are added, deleted or substituted in the amino acid sequence represented by SEQ ID NO: 1; a gene which encodes said protein; and a gene 10 which comprises DNA having the nucleotide sequence represented by SEQ ID NO: 1, or comprises DNA being capable of complementing the mutation exhibiting low-temperature-sensitive fermentability and having a nucleotide sequence wherein one or more DNAs are added, deleted or substituted in the nucleotide 15 sequence represented by SEQ ID NO: 1. The present invention also relates to yeast belonging to the genus Saccharomyces and having low-temperature-sensitive fermentability which is characterized in that the above-mentioned gene on the chromosome is inactivated; dough containing said yeast; a process for 20 making bread which comprises adding said yeast to dough; and a process for producing ethanol which comprises culturing said yeast in a medium, allowing ethanol to accumulate in the culture, and recovering ethanol from the culture.

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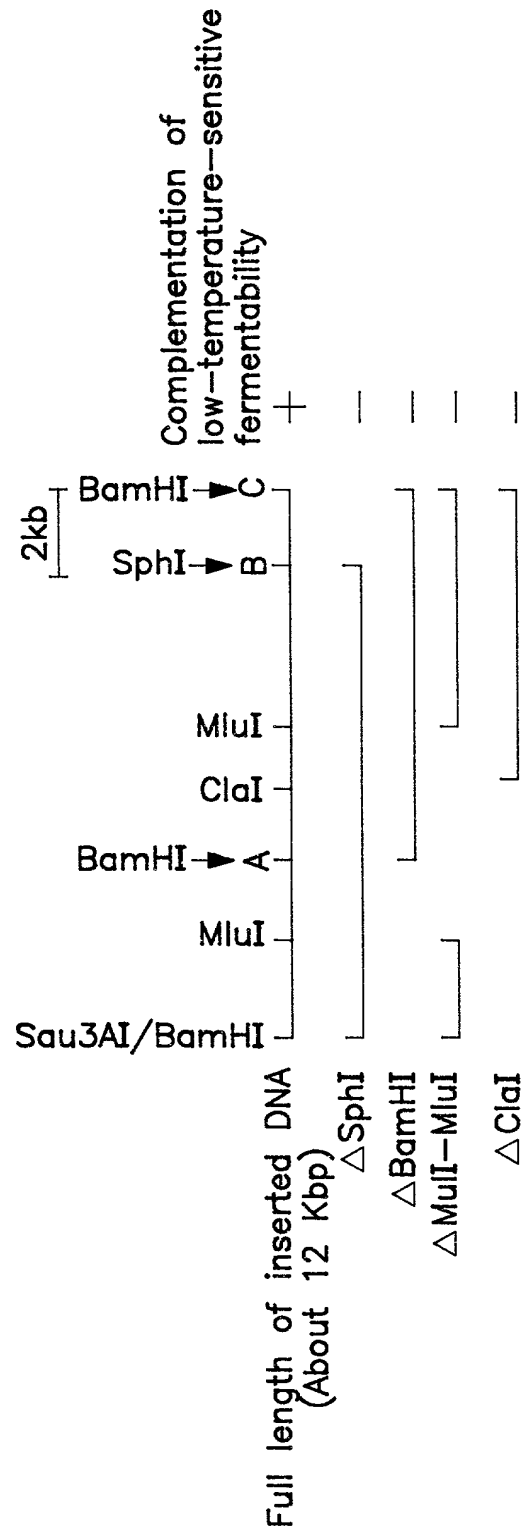


FIG. 1

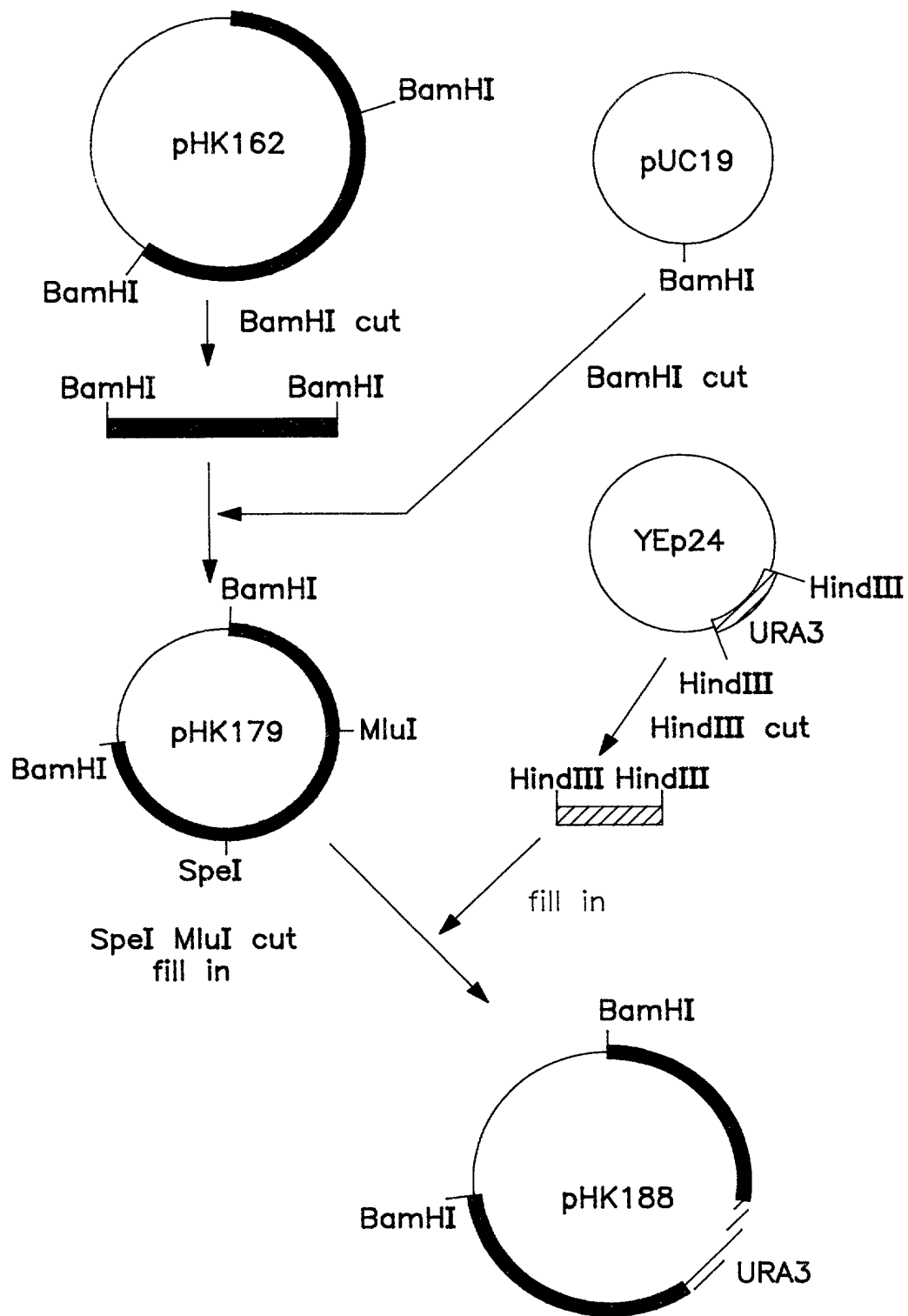


FIG. 2



COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR PATENT COOPERATION TREATY APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name;

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NOVEL YEAST GENE

the specification of which was filed as PCT international application No. PCT/JP96/03862  
on December 27, 1996 and was amended under PCT Article 19 on  
(if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35 U.S.C. §119(a)-(d) or §365(b), of any foreign application(s) for patent or inventor's certificate, or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate, or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

| <u>Country</u> | <u>Application No.</u> | <u>Filed (Day/Mo./Yr.)</u> | <u>(Yes/No)</u><br><u>Priority Claimed</u> |
|----------------|------------------------|----------------------------|--|
| JP             | 343700/95              | 28/12/95                   | Yes  |

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COMBINED DECLARATION AND POWER OF ATTORNEY  
FOR PATENT COOPERATION TREATY APPLICATION  
(Page 2)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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